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The Archives of Internal Medicine

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ON NONPARASITIC CHYLURIA*

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PITTSBURGH

Ever since the days of the celebrated Physician of Cos, fat in the urine was recognized and its significance discussed.[1] In his writings, Hippocrates speaks of several cases of fat in the urine, and mentions the case of a woman who several days after childbirth voided oily urine. It is curious to note that while many have written on parasitic chyluria, and some on nonparasitic chyluria, the chemical pathology of the latter variety is shrouded in as much mystery as it was centuries ago.

We have attempted briefly to review the literature on this subject, and those interested in the progress of medicine will find, we hope, the following summary of some value.[2]

After Hippocrates, who wrote of *fatty* urine and *oily* urine, Galen described three varieties of oleaginous urines, and gave unreasonable methods for their differentiation. The physicians that followed the Pergamite for many centuries added very little to his discussion of this subject. In the tenth century of the Christian era, the physician Theophile of Salerno in discussing lipuria, stated that the voiding of oily urine was indicative of approaching delirium, convulsions and death. The milky color of the urine was ascribed by many authors to the excretion of milk in the urine. Actuarius (thirteenth to fourteenth centuries A.D.), Gordon of Montpellier (*obit.* 1320 A. D.), and others taught that milky or fatty urine signified general febrile conditions and cachexia. Other physicians of the sixteenth century wrote that urine rich in fat was a symptom of hectic fever, phthisis and a general decomposition of the body and was a very grave indication of approaching death. In 1694 Peu of Paris reported that he had observed the excretion of milk in the urine of a parturient woman, and he thought that this was a normal means of eliminating excessive milk fat from the body.

* Submitted for publication Sept. 4, 1915.

* From the Biochemical Department of the Laboratories of the Western Pennsylvania Hospital.

1. Hippocrates: Aphorisms, lv, 7, No. 35.
2. Monvenoux, F.: Les Matiéres graisses dans l'urine, Paris, 1884.

The discovery in 1651 by Jean Pecquet of the circulation of lymph caused several authors to offer a different explanation for the appearance of chyluria. In 1670, Valentine Andrea Moellenbrogii wrote a treatise on Mictione Chylosa, in which he ascribed chylous urine to an abnormal junction of the urinary and lymphatic systems, but in his description he confused pus in the urine with chyluria, and it was only a century later that Morgagni clearly differentiated the two conditions and gave a scientific discussion of these symptoms.

Pierre Frank (1745-1831) reported a case of chylous diabetes. The patient, a man of 70, voided daily quantities of 16 to 20 liters of urine which much resembled milk. The patient had a very severe thirst and an insatiable appetite. The milky appearance of the urine was not due to pus.

Attempts at the scientific chemical investigation of lipuria began with the dawning of the nineteenth century. In 1817, Alibert[3] described a polyuria which he termed "caseous polyuria." We shall quote him verbatim:

> This variety is very rare. I have observed it in two cases. The urine in these cases is of normal daily quantity, and resembles very much milk upon which cream had accumulated. At that time I sent a considerable quantity to the celebrated chemist, Vauquelin, who was kind enough to submit it to a thorough chemical examination. He found in the urine substances which resemble fresh cheese and milk. This phenomenon can not be ascribed to lactation, for both of these women were advanced in years, and one of them never had any children.

The question as to the location of junction—i. e., where the chyle is admixed with the urine—is a mooted one. One group of authorities[4] assumes that the chyle or lymph passes by means of fistulous paths into the urinary system. A necropsy by Ponfick[5] seemed to corroborate this view, but this has been doubted. In a discussion before the Berliner medizinische Gesellschaft, Ewald expressed his opinion that the chyle united with the urinary system by means of fistulous connections. Virchow, however, at the same meeting, expressed his skepticism of this view, and said that it was difficult to explain anatomically such a condition of affairs.

Another group of investigators believes that the fat and albumin present in chylous urine are derived directly from the blood. They ascribe this condition to a disturbance of the functional activity of the kidney epithelium which permits of the passage of the abnormal con-

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3. Alibert: Nosologie naturelle, 1817, i. Fourcroy and Vauquelin: Ann. de chimie, 1800, xxxii, 82. Fourcroy: System des connaisances chim., 1801, v, 412.

4. Carter: Schmidt's Jahrb., cxx, 274. Ackerman: Deutsch. Arch. f. klin. Med., i. Dickinson: Trans. London Path. Soc., xxix, 391. Havelburg: Virchow's Arch. f. path. Anat., ix. Siegmund: Berl. klin. Wchnschr., 1884. Grimm: Ibid., 1885.

5. Ponfick: Schmidt Jahrb., clxix, 4.

stituents into the urine. They also found that the blood was very rich in fat in this disease.[6]

In 1884 Wilson[7] claimed that he had isolated an organism from the urine of patients suffering with chyluria which he thought was the causative factor of this disease. This observation has never been corroborated, and in fact many aspersions have been cast on the reliability of his findings.

In 1891, Wolff,[8] in his dissertation on this subject drew the following conclusions from his study of a case of chyluria and from the report of Goetze's findings:[9] Chyluria is certainly not due to disease of the liver. The secretion of chyle into the urine stands in no relation with fistulous passages between the lymphatic and urinary tracts. The fat and the albumin in the urine are directly excreted from the blood into the urine through the kidneys.

The references in this paper will show the number of investigators who have, mostly superficially, studied this condition.[10] We find that this disease has no predilection for either sex. The average age of the patients is about 40 years. No disease or condition of health seems to predispose to this disturbance. In fourteen cases reviewed by Monvenoux we find diabetes in one, bad alimentation in two, debility in two, and blennorrhagia, menopause, parturition, hemiplegia, chorea and lymphorrhagia in as many individual cases. Veis described a case in which chyluria was noticeable during pregnancy. The pregnancy was forcibly interrupted rather because of the excessive albuminuria than because of the chyluria. Chyluria may be unilateral as was reported by Israel[11] and others. Davis[12]

6. Eggel: Deutsch. Arch. f. klin. Med., xiv, 449. Thudichum: Schmidt's Jahrb., cxxiv, 270. Bence-Jones: Philosoph. Trans., 1850, v, 930. Brieger: Ztschr. f. physiol. Chem., iv. Cohnheim: Vorlesungen u. allg. Pathol., ii, 384.

7. Wilson: Lancet, London, 1884, p. 1128.

8. Wolff, H.: Zur Lehre von der Chylurie, Dissertation, Berlin, 1891.

9. Goetze: Die Chylurie, Jena, 1887.

10. Prout: Ann. Philosoph., xiii, 20. Nasse: Unters. z. Physiol. u. Path., 1835, p. 211. Bence-Jones: Med.-Chir. Trans., xxxiii, xxxiv. Waters: Ibid., 1862, 45. Begbie: Edinburgh Med. Jour., 1862, viii, 132. Beals: See Monvenoux (Note 2), p. 279. Morgan: Ibid., p. 285. Roberts: A Practical Treatise on Urinary and Renal Diseases, London, 1872, p. 327. Dickinson: Med. Times and Gaz., 1877, ii, 659. Ord, Miller: Trans Path. Soc., London, 1878, xxix, 402. Barbour: Glasgow Med. Jour., 1879, xi, 21. Bouchut: Gaz. d. hôp., 1879, lii, 847. Mehu: L'Urine, p. 221. Guyon: Traité clin. sur les Malad. des voies urin., Paris, 1881. Boissard: France med., 1882, xxix, 410. Brieger: Berl. klin. Wchnschr., 1885, p. 405. Olliver: Bull. Soc. D'Arthropolog., Paris, 1878, i, 44. Ackermann: Deutsch. klin., 1863, p. 23. Ralfe: Med. Times and Gaz., 1877, p. 571. Frank: Wien. med. Wchnschr., July 1, 1909. Salle: Deutsch. Med. Wchnschr., 1909, xxxv, 142. Haatek: Deutsch. med. Wchnschr., 1910, xxxvi, 794. Veis: Berl. klin. Wchnschr., 1905, xl, 27. Davis: Am. Jour. Obst. and Dis. Women and Child., 1913, lxviii, 3.

11. Israel: Mitteil. a. d. Grenzgeb. d. Med. u. Chir., 1903, ii, 171, 217.

12. Davis: Am. Jour. Obst. and Dis. Women and Child., 1913, lxviii, 3.

reported a case of chyluria in a pregnant woman. The patient had an uneventful pregnancy and was delivered at full term. The phenolsulphonephthalein test on the right and left kidneys showed that they functionated equally well.

Through the courtesy of Dr. Thomas T. Kirk of Pittsburgh we were enabled to make a careful study of the following case of chyluria. The history of the case will be found toward the close of this paper.

The patient, who had given birth to a child four months before this study was undertaken, complained of five main symptoms: Chyluria, polyuria, polydipsia, polyphagia and some loss of weight. These symptoms would make one think first of all of diabetes. The findings, however, have proved conclusively that this was not the case. The fact that she had lived for the past quarter of a century in Pittsburgh proper, and the fact that we could never find any parasite in her blood or urine, prove that this is a case of nonparasitic chyluria. The following is a report of the chemical and microscopical investigations that have been made on this patient.

THE URINE

Quantity.—The patient voided daily quantities of about 3,000 c.c. Latterly, however, the quantities daily voided have been less, the quantity fluctuating between 1,550 and 1,760 c.c. The patient declares that before she came to the hospital she was voiding very much more urine than she is doing now.

Color.—The urine varied in color from yellowish white to cream color. The separate specimens collected during the various hours of the day showed differing appearances. In the morning the urine was amber, but smoky; toward the afternoon the urines showed streaks of red. In general, the urine collected as a twenty-four hour specimen on several successive days resembled yellowish milk.

Odor.—The fresh urine was almost odorless or had the normal uriniferous smell. On standing for a few hours the urine became strongly ammoniacal, so that we used a few crystals of thymol to preserve the urine.

Specific Gravity.—The specific gravity varied between 1.005 and 1.011. The morning specimens had a higher specific gravity than the total twenty-four hours mixed sample.

Appearance.—The urine was entirely opaque. On centrifugalization a sediment of fibrin and blood appeared at the point of the centrifuge tube, but no lessening of the opacity resulted, showing that the emulsion was a very fine one and permanent. The urine did not change in appearance when allowed to stand in the ice box

for several days. White and reddish masses were observed in the twenty-four-hour specimens. These masses will be described under the heading *Protein*.

Reaction.—The reaction of the urine varied. It was usually neutral to litmus. However, on titrating the urine with decinormal sodium hydroxid, using phenolphthalein as an indicator, by Folin's method we found an acidity equal to 1.7 to 2.4 c.c. tenth normal sodium hydroxid for 10 c.c. of urine.

Filtration had no effect on the appearance or the color of the urine. But when the urine was shaken with ether and allowed to separate, it became amber in color, cloudy in appearance, but it had lost very much of its opacity. The urine after ether extraction was tested for albumin and found to be positive. After coagulation of the albumin and filtration, the urine was found to be clear, amber, and to all appearances looked like normal urine.

Protein.—The quantity of protein present in the twenty-four hour specimens varied. By Scherer's method the average figure obtained for three successive days was 3.1 parts per thousand. The Tsuchiya method gave a much lower figure of 2.7 parts per thousand. Solidified masses of fibrin and albumin were found in the urine. Some of these masses were almost pinhead in size and reddish in color; other masses were of the size of walnuts. No albumose was found. By fractional precipitation with ammonium sulphate both globulin and albumin were found.

Mucin.—Certain of the masses present in the urine had a gelatinous appearance, were stringy and gave tests for mucin. These mucin masses varied in quantity on different days. That these masses came from the urinary tract and were not admixtures from the genital passages is certain, for they were found in catheterized specimens.

Glucose.—The urine did not contain any glucose. Fermentation tests of the chyle urine and of the urine after the fat and the albumin had been removed gave negative results. The reduction tests were negative. No osazone could be formed. Tests for lactose, pentose and levulose gave negative results. No lactic acid was present.

Leucin, tyrosin, or *cystin* could never be demonstrated in any specimens of urine.

Traces of *indican* were always present.

Cholesterol.—The excretion of this substance varied considerably. In the first specimens of urine obtained from the patient some time ago the cholesterol was easily detected. Lately, however, cholesterol has decreased in the urine. The cholesterol was determined by the method of Weston and Kent.[13] The daily excretion varied in the

13. Weston and Kent: Jour. Med. Research, 1912, xxvi, 531.

early course of the disease from 0.4 parts per thousand to 0.85 parts per thousand.

Neither lecithin nor cholin could be detected in the urine.

Acetone, diacetic acid and beta-oxy-butyric acid were always absent.

Microscopic Appearance.—On examination of a centrifuged specimen, many red and white blood cells could be observed. Globules of fat of the size of ordinary milk globules were seen. These fat particles stained red with Sudan III. Triple phosphate crystals were usually present. Occasionally a crystal of cholesterol was observed. No granular or blood casts were ever noticed, though a thorough search was made for them. A few hyaline casts were usually present. Repeated painstaking examination for filaria or any other parasite proved futile. These examinations were made on specimens collected at different times during the twenty-four hours. Occasional squamous epithelial cells were seen. The bacterial findings were negative. Special examination for the tubercle bacillus gave negative results.

The elimination of nitrogen, sulphur and phosphorus was studied. The nitrogen partition reveals the fact that there is a large increase in the purin bases and creatinin excretion. This increase seems to be fairly constant. The ammonia nitrogen seems normal. The output of urea is decreased.

The accompanying table (Table 1) is the result of the determination of nitrogen partition on three successive days.

In the sulphur partition, which was done on the same days as the nitrogen partition, we found an increased neutral sulphur fraction. The total sulphur output seems normal. The inorganic sulphates are reduced, whereas the ethereal sulphates are also increased. Table 2 incorporates the results of this partition.

The phosphorus partition seems to be entirely normal so far as could be determined. The average daily excretion of phosphorus calculated as phosphorus pentoxid was 2.1084 gm. (analyses made on three successive days).

Not long ago, Young[14] had occasion to study the protein metabolism in two cases of chyluria. The first patient was a woman 42 years of age, who, though otherwise healthy, had noticed that her urine became milky in appearance, and clotted into jelly-like masses on standing. She had pains in her lumbar region which were dull and aching in character, and she had oberved that her urine was stained with blood. No parasites were ever found in the blood or in the urine. The second patient was a miner, 23 years old. For eight years he had been passing urine which was blood stained and which on standing became milky and had jelly-like masses. No filaria were

14. Young: Jour. Trop. Med. and Hyg., 1914, xvii, 241.

TABLE 1.—Nitrogen Partition in Urine

Day	Total N gm.	NH_2N Per Cent. Total N	Urea N Per Cent. Total N	Uric Acid N Per Cent. Total N	Purin N Per Cent. Total N	Creatinin N Per Cent. Total N	Rest N Per Cent. Total N	Quantity of Urine c.c.
1	14.7294	3.74	72.06	2.71	3.05	4.17	14.27	1,670
2	14.8107	3.42	73.37	2.14	3.17	3.82	14.04	1,980
3	12.9413	3.55	73.33	1.98	3.35	4.05	13.74	1,770
Average	14.1203	3.57	72.92	2.27	3.19	4.01	14.01	1,806

TABLE 2.—Urinary Sulphur Partition

Day	Total S. gm.	Inorganic Sulphate Per Cent. Total S.	Ethereal SO_4 Per Cent. Total S.	Neutral S Per Cent. Total S.	Volatile Sulphids	Quantity of Urine c.c.
1	2.107	38.4	23.78	37.82	Normal	1,670
2	2.291	36.7	23.86	39.44	Normal	1,980
3	2.088	32.5	30.79	36.71	Normal	1,770
Average	2.162	35.8	26.14	37.99	.....	1,806

found in this case. The fat content of the urine was 1.8 per cent in the first case and 2.6 per cent. in the second case. The quantity of protein nitrogen excreted daily varied in the first case from 0.49 to 1.94 gm., the average for the fourteen days of observation being 0.95 gm. per diem, corresponding approximately to 6 gm. protein. In general this patient showed a lowered nitrogen metabolism. In the second case the daily loss of protein nitrogen averaged 2.4 gm., equivalent to about 15 gm. protein. When, however, the diet was liberal and controlled only by the appetite of the patient, the quantity of nitrogen excreted decreased steadily, the nonprotein nitrogen falling as low as 6.1 gm. daily, corresponding to only 38 gm. protein catabolized, a figure below normal. The protein nitrogen excretion was still on the decline when the observations of the metabolism were discontinued.

Urinary Fat.—The quantity of fat varied in the daily specimens. In the early course of the disease it was much more than later on. Determinations by the Babcock method gave figures on successive days that fluctuated between 3.8 per cent. and 5.4 per cent. One evening sample yielded as much as 6 per cent. fat. Analysis by the Soxhlet method gave slightly smaller results, varying on various days between 3.2 per cent. and 5.1 per cent.

On shaking the urine with ether, the urine became demulsified and the ethereal extract was separated. The ethereal extract was of a canary yellow color. On driving off the ether a clear, limpid, yellow oil was obtained. It gave the characteristic "grease spot" on paper. Acrolein test was positive. The melting point of this crude oil was found to be 35.5 C. It was decided to extract a large quantity of fat and determine its composition.

The urine was dried with plaster of Paris and extracted with ether for seventy-two hours in a Soxhlet apparatus. The ether was then evaporated and the fat obtained. It was found that the fat was composed of two portions: One was soluble in alcohol and the other portion was not.

Alcohol soluble portion was obtained by evaporating the alcohol on a hot plate. An oil was obtained. It is soluble in warm alcohol, ether, chloroform, xylol, toluol and acetone. It separates out from the alcoholic solution when immersed in a freezing mixture. The oil is liquid at ordinary temperature. On immersion in a freezing mixture it solidified at —5 C. This oil was easily saponified by potassium hydroxid. The acid obtained by this method proved to be oleic acid, as determined by the melting point of the crystals (13.5 C.) and the Hübl iodin number. The acid was insoluble in water, but soluble in ether, chloroform and alcohol.

The alcohol insoluble portion is a white, opaque, greasy substance. It is solid at ordinary room temperature. While it is not soluble in cold or slightly warmed alcohol, it was found to be soluble in boiling alcohol. The fat crystallized on cooling the alcoholic solution.[15] The crystals obtained were rather irregular. They formed clumps from which projected long thin plates or needles. The melting point of several samples obtained on different days varied between 58 and 65 C. From its melting point and from its crystalline appearance we believe this fat to margarine. On saponification with potassium hydroxid we obtained crystals of fatty acids which resembled both stearic and palmitic acids.

Functional Tests of Kidneys.—Ureteral catheterization of both kidneys showed that fibrin clots and blood were present on the left side but absent on the right. This was not, however, a constant observation,

The accompanying comparison (Table 3) of the urines of the right and left kidneys is the result of one observation. Several other examinations with catheters at different periods gave other results (see later).

TABLE 3.—Comparison of Urines from Right and Left Kidneys

	Left Ureter	Right Ureter
Color	Amber yellow	Straw
Albumin	Heavy	Heavy
Reaction	Neutral	Acid
Fibrin	Present	Absent
Benzidin test	Positive	Negative
Red blood cells	Many	None
White blood cells	Many	Few
Casts	None	Few hyaline
Fat	Present	Present
Cholesterol crystals	Present	Absent
Ova and parasites	Absent	Absent

The Phenolsulphonephthalein Test.—This test was carried out twice on the patient. The first time was during the week when the patient was on a fat-poor diet. Both ureters were catheterized with the following results: It was found that the right kidney secreted during the same period five times as much urine as the left. The red color appeared in the right kidney in seventeen minutes, whereas in the left kidney the color was very faint and appeared only in twenty-six minutes. The amount of phthalein in the right

15. The cholesterol was separated from the oil by pipetting off the supernatant oil, which remains fluid at room temperature.

kidney (half hour's collection) was 3 per cent., and in the left kidney there was less than 1 per cent. The urine examined after ureteral catheterization of both kidneys gave the result shown in Table 4.

TABLE 4.—PHENOLSULPHONEPHTHALEIN TEST

	Right Kidney	Left Kidney
Phenolsulphonephthalein	3 per cent.	Less than 1 per cent.
Albumin	Very faint	Moderate
Fat	None	None
Red blood cells	None	Rarely
White blood cells	Rarely	Many
Casts	None	None
Epithelial cells	Few	Few
Parasites	None	None

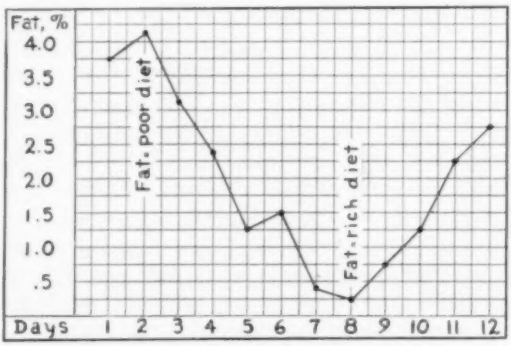
The phenolsulphonephthalein test was performed a second time during the period when the patient was on a fat-rich diet. The ureters of both kidneys were catheterized and the phenolsulphonephthalein collected. It was observed that the right kidney secreted in forty minutes 110 c.c. of urine, whereas the left kidney secreted only 6 c.c. Table 5 gives a comparison of the urine from the right and left kidneys.

TABLE 5.—COMPARISON OF URINES FROM RIGHT AND LEFT KIDNEYS

Before the Phthalein Test	Right Kidney	Left Kidney
Color	Yellowish	Watery
Appearance	Very cloudy	Faintly cloudy
Reaction	Acid	Acid
Albumin	Moderate	Heavy
Fat	Very much	Slight
Cholesterol	Present	Present
Glucose	None	None
Red blood cells	Many	Many
White blood cells	Many	Moderate
Epithelial cells	Many cuboidal	Many cuboidal
Casts	None	None
Parasites	None	None
After the Phthalein Test (Forty Minutes)		
Amount of urine collected	110 c.c.	6 c.c.
Phenolsulphonephthalein	8 per cent.	Less than 1 per cent.

Influence of Diet on Fat Excretion in the Urine.—The patient was kept on a diet poor in fat for a period of eight days, and it was found that the fat excretion was much reduced. The urine which was voided in the early morning was especially poor in fat,

was faintly cloudy and resembled very closely a urine with a moderate amount of phosphates. The amount of fat on two successive days in the twenty-four-hour specimen of urine collected on the sixth and seventh days that the patient was kept on this diet was 0.47 per cent. and 0.25 per cent., respectively. The accompanying curve shows the fat excretion on this diet. The amount of albumin excreted was also somewhat diminished. Blood in the urine was present only in small streaks.



Fat excretion on fat-free and fat-rich diets.

A diet rich in fat caused the following symptoms to appear in the urine. The early morning specimen was very cloudy, in fact much more cloudy than any individual specimen that was obtained during the previous course of the disease. The urine was milky and rich in fat, the morning specimen contained 2.14 per cent. on the third day, as determined by the Babcock method. Very much wax-like material was present as well as mucin.

In 1858 Thudichum[16] wrote about chylous urine as follows:

> The urine is oftener fatty when the patient subject to this disease lives on an animal diet than when he eats a more vegetable one. It is most clear before breakfast and most fatty after dinner. It is oftener free from fat before breakfast, when the diet is vegetable, than when it consists more of animal food. Fat passes off in the urine after food is taken, yet the albumin, fibrin and blood globules are thrown out before any food has been taken. During perfect rest the albumin ceases to be excreted, and it does not appear in quantity in the urine even after food is taken, provided there is perfect rest. The disease of the kidney permits fibrin, albumin globules and salt to pass whenever the circulation through the kidneys is increased; if at the same time fat is present in the blood, it escapes also into the urine.

THE BLOOD

An examination of the blood showed hemoglobin 78 per cent., leukocytes 5,400 and erythrocytes 4,200,000. Differential leukocytic

16. Thudichum: A Treatise on the Pathology of Urine, London, 1858, p. 240.

count gave the following average results: Polynuclears 77 per cent., lymphocytes 15 per cent., large mononuclears 7 per cent., eosinophils 1 per cent. No eosinophilia was ever found. The blood was searched on many occasions, and in specimens taken at different hours of the day, for parasites but none were found. The fat in the blood was only slightly increased. The urea in the blood was normal. Sugar was found to be 0.85 parts per thousand. The cholesterol content was very high—3.2 parts per thousand of blood serum.

THE FECES

The patient was usually constipated so that a laxative had to be administered. The feces thus obtained was soft, normal in color and odor. The fat content was quite normal. Bile pigment was present. Blood was absent. No parasites or ova could be found.

CLINICAL EXAMINATION OF PATIENT

Both kidneys were repeatedly catheterized. No obstruction was found. Cystoscopy and ureteroscopy gave negative results. Several Roentgen-ray examinations were made but nothing abnormal was found. There were no fistulous passages that could be found and no abnormaltiy in the kidney shadows.

An exploratory operation did not reveal any abnormality that was noticeable in the anatomy or situation of the kidneys. The blood vessels leading to and from the kidneys were normal. The ureters were normal.

HISTORY OF THE CASE

Mrs. A. S., housewife, aged 42, born in Germany. At the age of 13 she came to the United States, and with the exception of her first year's residence in New York, she has always lived in Pennsylvania. She has been married twelve years. Her menstrual history is normal. She has four children living. The oldest is 10 years and the youngest is 4 months old. Labors and puerperium were normal. Her family history is absolutely negative. About seven years ago her urine for a few months was of a reddish brown color, containing blood clots and occasional white curds. At that time she also complained of weakness and vertigo. Otherwise she has always been healthy. The present illness began on the third week after childbirth, about fifteen weeks prior to examination. She voids a milky urine of excessive amount; she has a ravenous appetite, and a sensation of emptiness in her stomach. She is continuously thirsty. She has lost considerable weight since the confinement. She has never complained of any obstructive pain in the urinary tract.

CONCLUSION

It would seem that this is a case of nonparasitic chyluria. Abundant evidence has been furnished that there is no pathologic structural connection between the lymphatic system and the urinary tract. It would appear that the chyluria in this case was due to a passage of fat from the blood directly into the urine through the kidney epithelium, caused, probably, by a functional disturbance of the kidney cells.

EXPERIMENTAL ALCOHOLIC CIRRHOSIS OF THE LIVER *

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Ever since the experimental method has been used in pathology numerous attempts have been made to show the connection between cirrhosis of the liver as often seen in "steady drinkers" and alcohol as the causative factor. In this experimental work alcohol has been used subcutaneously, intravenously, by inhalation, and fed by mouth. Ethyl alcohol has been used by most all, but some have used amyl and methyl alcohol, while others have used mixtures of these, and still others have used various beverages, such as schnapps, brandy and absinthe. Rabbits, guinea-pigs, dogs, rats, mice, hens, cats, ducks and pigeons have been used as experimental animals. Dahlström,[1] Duchek,[2] Joffrey and Serveaux,[3] von Baumgarten,[4] and de Rechter,[5] were among the first to carry out work along these lines, but with negative results. Ruge,[6] Magnan,[7] Kremiansky,[8] Mairet and Cambermale,[9] Afanassiew,[10] v. Kuhlden,[11] Friedenwald,[12] d'Amato,[13] Strass-

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1. Dahlström: Cited from Fahr, Note 23.
2. Duchek: Alkohol im tierisher Organismus, Prager Vierteljahrs, 1853.
3. Joffrey and Serveaux: Mensuration de la toxicite vraie de l'alcool, Arch. de méd. expér. et d'anat. path., 1897.
4. v. Baumgarten: Ueber die durch Alkahol hervorzurufenden pathologisch-histologischen Veränderungen, Verhandl. d. deutsch. path. Gesellsch., 1907.
5. De Rechter: Bull de l'Acad. roy. de méd. Belgique, 1892.
6. Ruge, L.: Wirkung des Alkohols auf den tierschen Organismus, Virchows Arch. f. path. Anat., 1870, xlix.
7. Magnan: Alcoolisme aigu, etc., Compt. rend. Acad. d. sc., 1869, p. 825.
8. Kremiansky, J.: Ueber Pachymeningitis interna hemorrhagica bei Menschen und Hunden, Virchows Arch. f. path. Anat., 1868, xlii.
9. Mairet and Cambermale: Compt. rend. Acad. d. sc., 1888, Nos. 10, 11 and 12.
10. Afanassiew, W. A.: Zur Pathologie des akuten und chronischen Alkoholismus, Beitr. z. path. Anat. u. z. allg. Path., 1890, viii.
11. v. Kuhlden: Cited by Fahr, Note 23.
12. Friedenwald: Pathological Effects of Alcohol on Rabbits, Jour. Am. Med. Assn., 1905, xlv, 780.
13. d'Amato: Ueber experimentelle, vom Magendarmkanal aus hervorgeru fene Veränderungen der leber und über die dabei gefundenen Veränderungen die ubringen Bauchorgane, Virchows Arch. f. path. Anat., 1907, clxxxvii.

man,[14] Pupier,[15] and Bischoff[16] in their early work at least got only parenchymatous degeneration of the liver cells as a result. Dujardin Beaumetz and Audige[17] fed hogs with their food, alcohol in the form of wood alcohol, malt spirits, beef spirits, potato spirits and absinthe. This experiment lasted three years, but owing to the large amount of interlobular connective tissue naturally present in these animals it was not a success

Straus and Blocq[18] fed rabbits ethyl and amyl alcohol by means of a stomach tube and obtained cirrhosis after three months. Pupier[15] claimed that experimenting with hens he got an interstitial hepatitis with absinthe, hypertrophic cirrhosis with red wine and atrophic cirrhosis with white wine. Afanassiew[10] gave dogs, rabbits, guinea-pigs, and white rats, by means of a stomach tube, a mixture of ethyl and amyl alcohol, and in some instances obtained a well marked cirrhosis. He also injected alcohol into the portal vein of dogs with positive results. De Rechter[8] got similar results. Mertens[19] obtained cirrhosis in rabbits by keeping them for a long time in an atmosphere containing alcohol vapor. Joannovics[20] successfully repeated this experiment but Challand[21] and Magnan[7] were unable to do so. Saltykow[22] injected absolute alcohol in physiological salt solution in the ear vein of rabbits. One animal lived almost two years, receiving in this time eight injections, each containing 2½ c.c. of absolute alcohol. He found a resulting cirrhosis that was well marked macroscopically and microscopically.

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14. Strassman, F.: Experimentell Untersuchungen zur Lehr. vom. chronischer alkoholismus, Vrtljschr. f. gerichtl. Med., 1888, xlix.

15. Pupier, Z.: Demonstration expérimentale de l'action des boissons dites spiritueuses sur le boie, Compt. rend. Acad. d. sc., 1872, p. 1415.

16. Bischoff: Neue Beitrage zur experimentellen Alkoholforschung mit besonderer Beruchsichtigung der Hertz und Leberveränderungen, Ztschr. f. exper. Path. u. Therap., xi, Part 3, p. 445.

17. Dujardin Beaumetz and Audige: Recherches expérimentales sur la puissances toxique des Alcools, Paris, 1879; Recherches expérimentales sur l'alcoolisme chronique, Paris, 1884.

18. Straus and Blocq: Etude expérimentale sur la cirrhose alcoolique de foie, Arch. de physiol norm. et path, 1887, Series 3, x, 409.

19. Mertens: Lésions anatomiques du foie du lapin au course de l'intoxication chronique par le chloroforme et par l'alcool, Arch. de pharmacod. et de therap., 1896, ii.

20. Joannovics: Recherches expérimentales sur le pathogenie de l'ictere, Mém. couron. Acad. roy. de méd. de Belg., 1903; Ueber Veränderungen der Leber bei Vergiftung mit Karbaminsäuren und kohlens Ammoniak, Arch. de pharmacod., 1903, xii; Ueber experimentelle Lebercirrhose, Wein. klin. Wchnschr., 1904, xvii, 25.

21. Challand, T.: Etude expérimentale et clinique sur absinthisme et l'alcoolisme, Thése de Paris, 1871.

22. Saltykow, S.: Beitrag sur Kenntnis der durch Alkohol hervorgerufenen Organveränderungen, Verhandl. d. deutsch. Gesellsch., 1910, xiv; Experimentelle Forschung über die pathologische Anatomie des Alkoholismus chronicus, Zentralbl. f. allg. Path. u. path. Anat., 1911, xxii, No. 19.

Fahr[23] repeated this work using 96 per cent. alcohol on two rabbits and two guinea-pigs for a time period of two years and got a beginning cirrhosis in one rabbit. Lissauer[24] used 50 per cent. alcohol and brandy in the ear vein of rabbits, and after seven months got evidence of cirrhosis. Schafir[25] reported success with similar experiments. Pogenpohl,[26] Siegenbeck von Heukelom,[27] Klopstock,[28] v. Baumgarten[4] and Bischoff,[16] working along the same lines, got merely fat degeneration. Lancereaux[29] conducted experimental researches for years on cirrhosis of the liver, particularly the type that is seen in France, and as a result he concludes that alcoholic cirrhosis of the liver is the result of substances used in wine, beer, etc., for their preservation, especially potassium bisulphate. He was able to produce typical lesions of "gin-liver" by feeding animals this salt. The other salts of potassium gave negative results. Kyrle and Schopper[30] used a 50 per cent. solution of absolute ethyl alcohol in equal parts of water and physiological salt solution intravenously, subcutaneously and by mouth on a series of thirty-one rabbits. The experiment extended over a period of from one hour to thirteen weeks. Each animal received alcohol every second to fourth day, in the dosage of 1.8 c.c. of absolute alcohol per kilo. In all cases the liver showed bile stasis, parenchymatous degeneration and fatty change in the liver cells. These changes commenced about the central veins. In seven cases there was necrosis of portions of the lobe and replacement fibrosis. In fourteen cases there was round cell infiltration of the interlobular spaces, and in seven others connective tissue proliferation with bile duct formation. The livers of three of the rabbits showed typical Laennec's cirrhosis. The authors state that degeneration only could be shown during the first two weeks of the experiment, but after that time the connective tissue reaction could be observed.

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23. Fahr: Zur Frage des chronischen Alkoholismus, Verhandl. d. deutsch. path. Gesellsch., 1909, xiii; Diskussionbemerkungen, ibid., 1910, xiv; ibid., 1911, xv; Virchows Arch. f. path. Anat., 1911, ccv.

24. Lissauer: Lebercirrhose bei experimenteller Intoxication, Virchows Arch. f. path. Anat., 1914, ccxvii, Part 1, p. 56.

25. Schafir: Virchows Arch. f. path. Anat., 1913.

26. Pogenpohl: Zur Frage der Veränderungen des Pankreas bei Lebercirrhose, Virchows Arch. f. path. Anat., 1909, cxvi.

27. Van Heukelom Siegenback: Die experimentelle Cirrhosis hepatitis, Beitr. z. path. Anat. u. z. allg. Path., 1896, xx.

28. Klopstock, F.: Alkoholismus und Lebercirrhose, Virchows Arch. f. path. Anat., 1906, clxxxiv; Zur Leber von der Lebercirrhose, Berl. klin. Wchnschr., 1910, Nos. 33 and 34.

29. Lancereaux: Granular Cirrhosis of the Liver, Bull. de l'Acad. de méd., Paris, 1910, lxxiv.

30. Kyrle and Schopper: Virchows Arch. f. path. Anat., 1913, ccxv, Part 2, p. 359.

In a previous communication I[31] made a preliminary report of work on experimental cirrhosis. Two of the cases reported were on experimental alcoholic cirrhosis and are as follows:

Experiment 4.—A rabbit was given by mouth daily, except Sunday, before feeding-time in the morning, 15 c.c. of 34 per cent. alcohol. This was given with a medicine dropper, and after a few administrations no difficulty was found in the rabbit's swallowing it. By this method I endeavored to reproduce as closely as possible the conditions in the steady drinker that are supposed to predispose to cirrhosis of the liver, namely, a repeated ingestion of alcohol in the "whisky proportion" on an empty stomach. This experiment was started Nov. 4, 1912, and the animal killed April 9, 1913, covering a period of slightly over five months. Necropsy showed a liver small for an animal as large as this one (which at the beginning was a full sized rabbit, and which

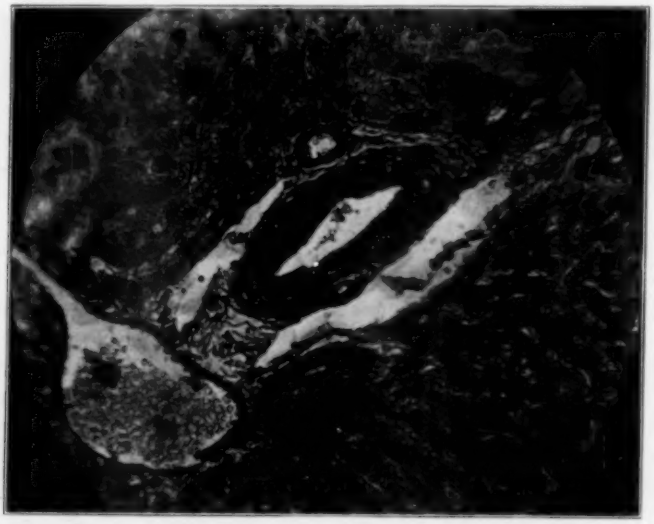

Fig. 1.—Normal rabbit liver showing normal interlobular space with very slight amount of connective tissue between blood vessels and bile ducts.

grew fatter and larger in the meantime, never showing any signs of being sick). The liver markings were extremely prominent and the organ was lighter in color and firmer; there was some slight roughening of the surface. Histologically, sections showed quite a large increase in the interlobular connective tissue in which there were many proliferating bile ducts, the buds and new branches of which stood out very distinctly. In several places it was evident that these were trying to penetrate the periphery of the lobules. The blood vessels were not particularly noticeable. The connective tissue seemed to be proliferating from the preexisting connective tissue in the interlobular

31. Grover, A. L.: Experimental Cirrhosis of the Liver, Jour. Am. Med. Assn., 1913, lxi, 458; The Question of Spontaneous Cirrhosis of the Liver in Rabbits and Other Laboratory Animals, Jour. Am. Med. Assn., 1915, lxiv, 1487.

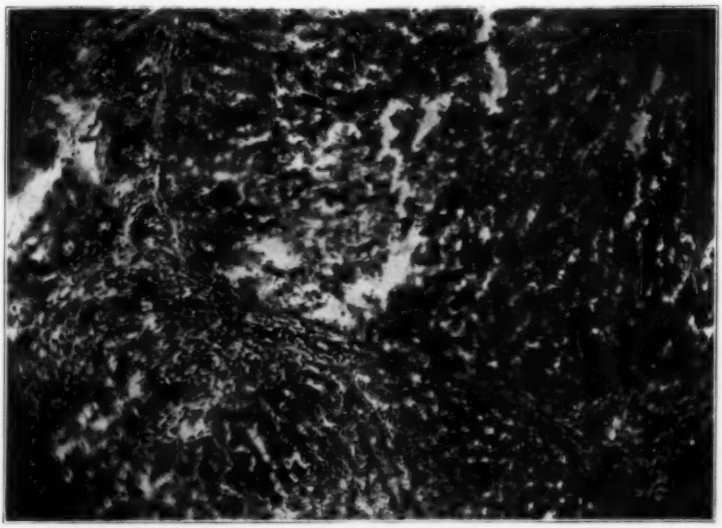

Fig. 2.—Showing a moderate cirrhosis (Animal 11) with areas of degeneration particularly at periphery of the lobule.

Fig. 3.—Marked cirrhosis (Animal 7); shows dense proliferation of connective tissue in interlobular spaces with many new formed bile ducts.

spaces. Some ingrowth into the lobule had taken place, but was not particularly far advanced. More or less increase of the intralobular connective tissue was also observed. The cells of the liver parenchyma showed pretty much throughout a moderate degree of albuminoid degeneration. Occasionally the central part of the lobule and in one place a whole lobule had undergone a more severe change, but not far enough to be called a true necrosis. With the higher power of the microscope the protoplasm of the cells has the lattice-work arrangement of threads as described previously. These threads where they crossed one another seemed to be sort of bunched up in a way similar to the nodal points in fibrin fibers. The protoplasm of the cells had a tendency to gather at the periphery of the cell along the cell membrane, and these threads intervened across the more central portion. The nuclei stained rather irregularly, having somewhat of a similar thread-like appearance of its chromatin. Karyokinetic figures were rarely observed, but a good many cells were seen

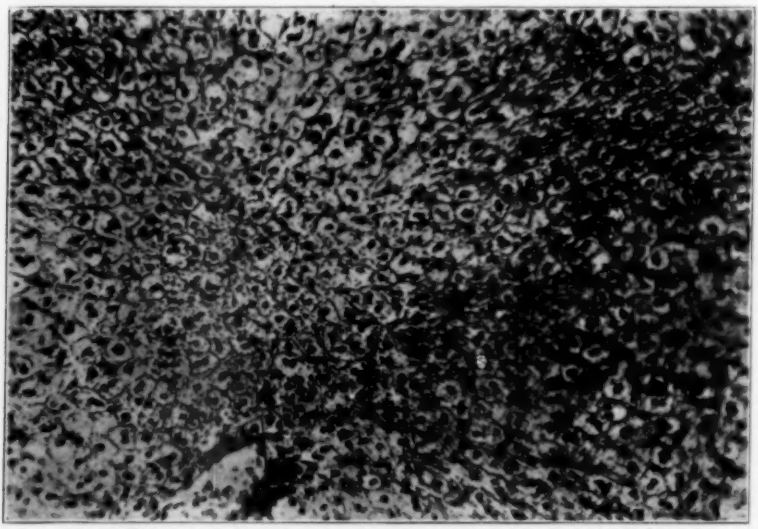

Fig. 4.—Complete parenchymatous degeneration with vacuolation (Animal 12). Nuclei appear broken up or have entirely disappeared.

with double nuclei. In some cells the nucleus was apparently lost, yet the protoplasm had the characteristic appearance already noted. An occasional cell was vacuolated, but nothing could be determined as to the contents of the same. Once in awhile a cell or a group of cells would stain more brightly than the others. As some of these had double nuclei, I took them to be regenerated or regenerating cells. Very rarely in the protoplasm of a cell could be made out a portion, really one of these nodal masses mentioned before, that took a brighter stain with eosin than the others. This may be the beginning of that hyaline skein-like degeneration that Mallory believes to be pathognomonic of alcoholic cirrhosis.

The intralobular blood capillaries were dilated and their walls could be fairly well made out. The endothelium of these as well as that of the lymph spaces in the portal regions was actively proliferating.

Experiment 5.—Another animal was treated in the same way for four months, but at the end of the third month was inoculated with 0.5 c.c. of a bouillon culture of *B. coli* in the ear vein. Histologically the liver shows a beginning of cirrhosis but much less than the last. The bile ducts have not yet undergone any change, thus showing that bile-duct proliferation is a later process than the proliferation of connective tissue. The colon bacilli seemed to have no influence, thus acting contrary to the results obtained with it in chloroform necrosis.

Since this report was made another series of experiments have been carried out all on rabbits. In all, twelve animals have been fed alcohol in same dosage as in the first experiment. Six other rabbits, four from same litters and two from other litters, have been saved for controls. In brief these experiments resulted as follows:

Fig. 5.—Extensive cirrhosis (Experiment 4); the interlobular connective tissue proliferation is well marked, but new formation of bile ducts is the most prominent feature.

Animal 1.—Lived eight months. Liver shows no gross changes but microscopically one sees areas of degeneration, particularly in the periphery of the lobules, but in some areas extending in as far as the central vein. In one or two lobules there seems to be complete necrosis of cell protoplasm with vacuolation. The interlobular spaces are filled with proliferated connective tissue, which is commencing to invade the periphery of the lobules.

Animal 2.—Lived five months. Examination of liver showed passive congestion with some central necrosis. All the trabeculae seemed to be widely separated. There is some slight connective tissue increase in the interlobular spaces.

Animal 3.—Lived four months. Liver showed nothing except some fatty degeneration.

Animal 4.—Lived three weeks. Liver showed no change.

Animals 5 and 6 were controls. One lived nearly a year and then died from pneumonia. Liver showed no lesions. The second control is still living after thirteen months.

Animal 7 lived about ten months. Shows a marked cirrhosis.

The interlobular spaces throughout show very marked proliferation of connective tissue which is crowding in on the lobules, very profusely invading the parenchyma cells, which are more or less degenerated.

Animal 8 lived eight months. Showed a moderate degree of cirrhotic changes about half way between that of Animals 1 and 7.

Animal 9.—Control. Lived eleven months and then was killed by a dog that broke into the pen. Showed no liver lesions.

Animal 10.—Lived six months; showed marked beginning cirrhosis.

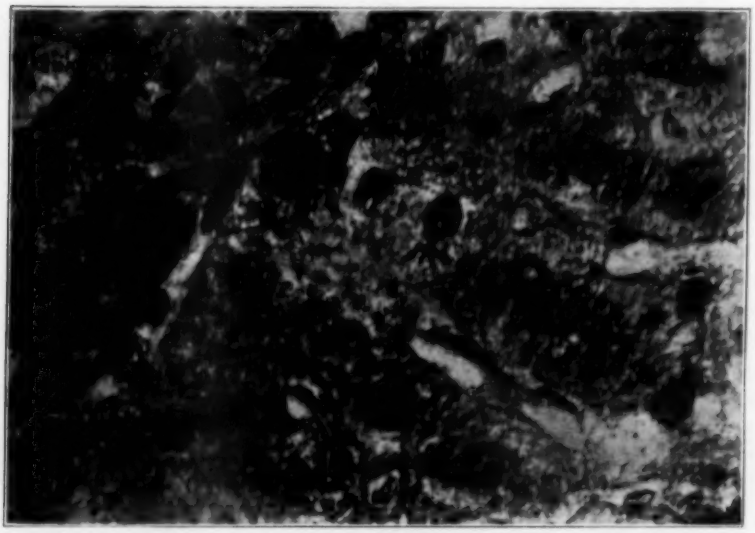

Fig. 6.—High power of liver parenchyma from same slide as Figure 5. Shows the skein-like formation of protoplasm with masses undergoing hyaline degeneration.

Animal 11.—Lived nine months; showed considerable connective tissue reaction. A rather marked cirrhosis with considerable bile duct proliferation. A good deal of parenchymatous tissue seems to have disappeared and to have been replaced by connective tissue. The newly-formed bile ducts can be seen running in the midst of this dense fibrous tissue.

Animal 12.—Animal killed after about twelve months on account of a severe long-standing torticollis. After nine months, the dose of alcohol was doubled. After two months more it was again doubled. Necropsy showed a pale, soft, enlarged liver. Microscopically, the cells are completely degenerated. Nothing remains but a vacuolated cell with a broken up nucleus. In many instances the nucleus had disappeared.

Animal 13.—Lived ten months. Had to be killed, as overgrowth of lower teeth resulting from broken upper ones prevented animal from eating. Liver

showed enormously enlarged blood vessels and capillaries and quite a marked cirrhosis that appeared to be originating entirely from the blood vessels and in many places it was somewhat of the nature of an adventitial sclerosis.

The rest of the animals and controls are still alive. It is interesting to note that almost all the young born of females treated with alcohol were killed by the mother while those from the controls lived. From the work of previous writers, as well as from this work, it appears that the cirrhotic change is entirely a reparative process stimulated by injury to the liver cells. The injury of the cell is manifested by albuminoid and fatty degeneration, but also particularly by vacuolation. At the same time there seems to be a gathering of the protoplasm into the skein formation previously mentioned, where it appears to be

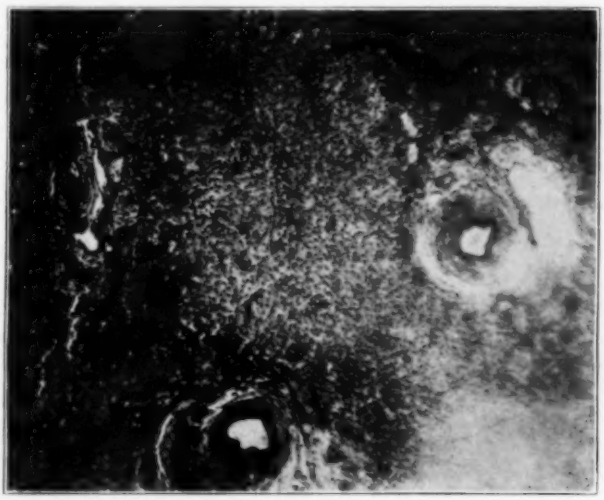

Fig. 7.—Showing a very early cirrhosis. The new formed connective tissue is seen proliferating from the region of bile ducts. Animal 10.

taking on a hyaline appearance very much like that which Mallory mentions as the diagnostic sign of alcohol degeneration. In all cases active regeneration of liver cells can be observed.

Some observers have claimed that spontaneous cirrhosis of the liver is a common condition in rabbits. This question has been fully considered in a previous contribution.[31] The evidence presented in this contribution was briefly as follows:

Nearly three hundred rabbits had been necropsied as a routine laboratory procedure, and these were used as controls for the foregoing experiments with alcohol. No evidence of cirrhosis of the liver was found except in one case, in which the common bile duct was

experimentally ligated, in one case of experimental pyemia, in one case of experimental chloroform intoxication, in three cases in which the rabbits died as a result of spontaneous pyogenic infection, and finally, in one case of infection with *Coccidia oviforme*. These animals were of the same strains as the ones used in the alcohol experiments together with many other strains, thus giving as fair a control as would be possible for one to find. As a result there has been no evidence found of spontaneous cirrhosis of the liver. The evidence brought forward by others is of interest, but I believe that a more careful examination would have shown evidence of infection of some sort as the source of the connective tissue proliferation.

In conclusion, it seems that when alcohol is given for a long period there follows degeneration of the liver cells, which in a large proportion of cases is followed by connective tissue proliferation. Whether any other process comes in to help is uncertain; nevertheless it remains pretty well proved that a cirrhosis can be obtained from alcohol in cases in which no other factor is apparent.

THE BLOOD AND THE BLOOD VESSELS IN HEMOPHILIA AND OTHER HEMORRHAGIC DISEASES *

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It is becoming increasingly evident that the group termed "the hemorrhagic diseases" includes a large number of abnormal conditions, and that, at the present time, it is a fruitless task to attempt to unravel the various entities embraced by the clinical conditions which are assembled under this general head. This is due partly to the fact that the physiology of the coagulation of the blood is still incompletely understood, partly because of the impossibility of analyzing the various factors concerned in coagulation, and in part because these hemorrhagic states have been incompletely observed from a clinical point of view. Indeed, the knowledge and the methods which are at our disposal have by no means been fully and intensively applied to solving the problems which these cases present.

There are two hemorrhagic states which stand out in relief—true hemophilia of the hereditary type, found almost exclusively in males and transmitted through the female, and on the other hand, the purpuric conditions, embracing many varieties and found quite as frequently among females as among males. Under this latter heading are included purpura hemorrhagica, Henoch's purpura, simple purpura, the purpura of the new-born, and many other forms which have received more or less wide recognition. It would evidently be impossible to attempt to consider these multitudinous disorders, and, we may add, would be to no purpose and lead to no definite conclusions. A brief consideration of the clinical manifestations which are supposed to distinguish these conditions one from the other is convincing that sharp boundary lines do not really exist and that at present no satisfactory differentiation can be drawn. We shall therefore consider purpura rather as an entity and compare it to hemophilia. Miss Mildred Fish has assisted in a large part of this investigation.

During the past year or more we have had the opportunity of studying the blood and observing clinically several cases of typical hemophilia, as well as some atypical instances of this disorder, and numerous cases of purpura. The opportunity has been unusual in that many of the children have been inmates of the Hebrew Infant

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Asylum, which rendered it possible to watch them clinically for a long and continuous period, and to carry out a large number of examinations of their blood. These tests included as a routine the determination of the coagulation time and of the amount of antithrombin present. In testing the coagulation of the blood and plasma, Howell's method[1] was followed. For the plasma, this consists of mixing the blood, which is obtained directly from the vein, with 10 per cent. of a 1 per cent. sodium oxalate solution. This mixture is centrifugalized for fifteen minutes and the supernatant plasma is pipetted off and recalcified with varying amounts of a weak calcium solution. In addition, frequent counts of the blood platelets were made by means of the Wright and Kinnicutt method, and many tests which will be referred to in the body of the paper.

TABLE 1.—VARIATIONS IN COAGULATION OF NORMAL PLASMA. (A. F. H.)

Date	Coagulation Time minutes	Remarks
1914		
March 24.....	3	Antithrombin test (Howell) showed a deficiency of this substance.
April 28.....	8	
April 30.....	4	Coagulation time of blood six minutes.
May 26.....	4	
June 15.....	10	
June 19.....	12	
Aug. 26.....	8	
Sept. 11.....	11	Coagulation time of blood September 3 was six and one-half minutes.
Oct. 21.....	10	

The pathogenesis of true hemophilia is still obscure. Some, following Howell, believe that the defect lies in an insufficiency of prothrombin in the blood; others, following Morawitz, attribute the excessive bleeding to a lack of thrombokinase. There is, however, concordance in the belief that the main defect lies not in the blood vessels, but in the blood itself. Normal plasma tested after the manner just described, forms a firm clot in about ten minutes. (Table 1.) The plasma of the hemophiliac has a delayed clotting time, varying generally from about one-half hour to many hours. The accompanying table (Table 2) shows numerous tests of plasma in several cases of hemophilia in patients who gave a classical hereditary history.

The first two are brothers (Hyman and Julius S.). There is one other child in the family, a girl of about 12 years. She is perfectly well and had her tonsils removed without hemorrhage. The father's family history is negative.

1. Howell, W. H.: THE ARCHIVES INT. MED., 1914, xiii, 76.

TABLE 2.—Coagulation Time in Hereditary Hemophilia Cases

A. HYMAN S. (5 YEARS OLD)

Date	Coagulation Time Plasma minutes	Coagulation Time Blood minutes	Remarks
1914			
March 11...	25	.....	Platelets 197,000
March 20...	10	.....	
March 24...	15	.....	
April 21...	.....	.....	Did not clot.
May 26.....	24	.....	Bleeding from tongue April 25 to May 2.
June 1.....	20	22	
June 8.....	20	.....	
June 26.....	10	.....	No loss of blood, hemarthrosis of knee.
Sept. 16.....	16	13	
1915			
June 12.....	8	.....	Severe hemorrhage of tongue stopped by thromboplastin.
June 19.....	12	.....	

B. JULIUS S. (9 YEARS OLD)

1914			
May 5.....	36	61	Hemarthrosis of ankle.
May 26.....	16	.....	Platelets, 300,000; leukocytes, 17,000.
Aug. 26.....	19	.....	In hospital, bled five days after incisor tooth extraction.
Sept. 14.....	42	.....	
Nov. 24.....	.....	35	
Dec. 11.....	.....	34	
Dec. 14.....	.....	60	
Dec. 16.....	.....	20	Bleeding from finger all day.
Dec. 17.....	.....	34	
Dec. 21.....	.....	48	

C. WM. B. (7 YEARS OLD)

1914			
Nov. 13.....	52	90	Plasma of brother (9 years old) clotted in nine minutes.
Nov. 20.....	20	55	
Nov. 24.....	.....	106	
Dec. 16.....	100	210	Five days ago severe bleeding from tongue.
1915			
June 14.....	24	.....	Plasma of brother (20 years old) clotted in twenty-six minutes.

The mother had no tendency to bleeding and had a peritonsillar abscess opened without great loss of blood. Her two sisters' children are also bleeders. One sister lost two boys from hemorrhage; one of these died following an injury to the mouth which broke several teeth, the history of the other is not definite. Another child (a girl) is supposed to bleed severely from cuts, so that a physician has frequently to be called. Another sister (an aunt of Hyman and Julius) has a daughter (Yetta K.) who is a bleeder and who will be discussed at greater length. As for the boys themselves, they have been in the Hebrew Infant Asylum for a number of years, have had frequent hemorrhages into the joints (ankle, knee, vertebrae), have bled from the tongue several times so as to necessitate their removal to a hospital, and are at no time free from large "black and blue" marks. Julius had to be taken to a hospital when ten days old, on account of hemorrhage following circumcision. At 2½ years of age he was transfused at a hospital on account of loss of blood.

Wm. B. is an Italian with a classical history. He is one of twelve children; the girls are normal; one boy died of hemorrhage, another is absolutely free from this tendency and has a normal coagulation time, a third is a bleeder and as a result is crippled with stiffening of the elbow and knee joints. The maternal uncle of the boy died at the age of 18 of hemorrhage. There are two maternal aunts: one had eight sons, seven of whom were hemophiliacs; the other had five sons, of whom four were hemophiliacs.

A glance at the column (Table 2) giving the clotting time of the plasma shows that, compared to the normal, it was almost regularly delayed. The first two cases, the brothers Hyman S. and Julius S., must be considered rather mild examples of hemophilia, in spite of the fact that at all times they manifested subcutaneous hemorrhage, and from time to time suffered from prolonged and serious hemorrhage.[2] The variability in coagulation time, which is a striking feature, has been found by various investigators to be the rule rather than the exception in hemophilia, although the cause of these variations has never been explained. The two tests of Hyman S., March 20 and January 26, are particularly interesting and noteworthy, because the coagulation time was only ten minutes, that is, was well within normal limits. This rapid clotting was not occasioned by previous hemorrhage (a phenomenon first noted by Sahli), nor by an admixture of tissue juice. These tests mean that had we depended on the clotting time of the blood on these two occasions for a diagnosis of the nature of the hemorrhagic condition, we should not have regarded the case as one of hemophilia. It emphasizes the necessity of numerous coagulation tests in every case. In spite of these occasional variations, the fact holds true that a marked delay in the coagulation of the blood is the most characteristic sign of hemophilia.

Another important element in diagnosis is the number of blood platelets. Our experience confirmed that of Duke[3] and others, that

2. The elder of these boys bled for several days following an injury to his tongue, and is the case referred to in an article on "Tissue Extract as a Hemostatic," where the local application of thromboplastin brought about almost instantaneous clotting. (Jour. Am. Med. Assn., 1915, lxiv, 1395.)

3. Duke, W. W.: The Archives Int. Med., 1912, x. 445.

in hemophilia the platelet count is normal, whereas in purpura it is low, frequently less than 100,000. In our cases of hemophilia the platelets never fell below 320,000 per c.mm., and in some tests numbered over 500,000. In purpura, on the other hand, the counts never exceeded 200,000. These cells have recently been the object of particular attention, as they contain a considerable amount of thromboplastic substance, the thrombokinase of Morawitz, and it seemed as if their study might shed new light on the pathogenesis of hemorrhagic conditions. Examinations of many cases have convinced us that the microscopic appearance of platelets is not always the same, that from a morphologic standpoint we can in some instances differentiate and distinguish between them. In one case of marked purpura, on which we carried out many platelet counts and numerous other tests, the platelets, on two occasions, were exceptionally large. In another instance the platelets were unusually small, appearing almost fragmented. It is probable that in future it will be well to note these *macro- and micro-platelets,* just as we are accustomed to take cognizance of the macrocytes and microcytes among the other blood cells.

Following puncture of the skin by a hypodermic needle, we should expect in hemophilia an exaggerated degree of bleeding, at least a small areola of hemorrhage, about the puncture wound. Such, however, is not the case. In one of our cases (H. S.), who developed diphtheria, numerous subcutaneous injections of antitoxin were given and not only did these inoculations not result in any marked hemorrhage, but there was not the slightest extravasation at the site of the injection. This circumstance and the fact that we can carry out venepuncture with impunity, is probably attributable to the thromboplastic activity of the tissue juices which continue to exert their complementary coagulative function in this disorder.[4]

This lack of hemorrhage following a puncture of the skin is sufficiently constant to be accorded some diagnostic significance, especially as quite the contrary reaction is occasioned in purpura, a marked hemorrhagic area manifesting itself at the site of puncture.

As has been shown by Duke,[3] the bleeding time is increased in purpura. By this is meant that bleeding does not stop as promptly as in the normal individual, but that the blood wells forth drop by drop for a considerable period. The test is extremely simple; a prick is made with a needle into the lobe of the ear and the drops of blood

4. The accompanying illustration shows a remarkable clot which formed on the tongue of a hemophiliac (Julius S.) at the site of hemorrhage. The clot was evidently held firm at its rim by the complementary action of the tissue juice, whereas the hemorrhage continued to some degree in the center. As a result, a cylindrical clot, fully one-half inch in length, developed. This clot, which resembled a papilloma, checked the bleeding for some days.

are absorbed with blotting paper. If the blood oozes for more than three minutes, the bleeding-time is considered increased. The test is of value in a positive sense, namely, if the time is found to be prolonged. The results vary considerably even from apparently identical punctures made at the same time. In hemophilia, the bleeding-time is generally normal. That this is so can be attributed probably to the same complementary factor as the normal "puncture test."

Some time ago we described a test, termed the *"capillary resistance test,"*[5] which is carried out as follows: A tourniquet is applied to the upper arm sufficiently tight to obstruct the venous flow, but not to completely obliterate the radial pulse. In infants, on whom this test was extensively carried out, pressure of about 100 mm. of mercury is required for the purpose. This pressure is maintained for three minutes, during which period, if it is properly applied, the forearm should become markedly cyanosed. The tourniquet is then removed

Blood clot following hemorrhage of tongue in hemophiliac.

and we note whether a large number of petechiae or small hemorrhagic spots have developed on the forearm. This test has been found useful in differentiating between purpura and hemophilia. It is surprising the degree of pressure which the blood vessels withstand in hemophilia without the formation of minute hemorrhages. In purpura, on the other hand, as must have been noticed by those who have applied a tourniquet in the course of aspirating blood, the application of the tourniquet quickly brings about not only petechiae, but even larger subcutaneous hemorrhages.

Let us turn now to hemophilia. It is common knowledge that this disorder has shown itself to be of most marked hereditary character, and that this heredity is peculiarly sex-linked, being transmitted by the female and manifested by the male. This law of heredity, which is sometimes termed the law of Nasse, runs so true that many have

5. Hess, A. F., and Fish, Mildred: Am. Jour. Dis. Child., 1914, viii, 386.

believed that it is without exemption, and have cast aside as unconvincing or unauthentic all cases which seem to run contrary to it. Let us consider this question; but, first of all, the simple one of hereditary bleeders.

The striking hereditary character of true hemophilia has frequently led to the assumption that the mere fact that the bleeding tendency was hereditary constituted strong evidence for the hemophiliac and against the purpuric nature of the disorder. This has been our experience in regard to cases referred to us by physicians, and is the point of view which pervades the literature of the hemorrhagic diseases. The matter, however, is not so clear cut. The fact is that, although purpura may not be hereditary, and may be idiopathic or due to sepsis or many other causes, there is a definite *hereditary purpura*. Whether this form of purpura differs from other forms in respects other than that it is not acquired, we are unable to state, but the hereditary nature of the bleeding seems no more open to question than the heredity of true hemophilia. Such being the case, it would seem advisable, from a clinical standpoint, to recognize and to emphasize this fact, and to make a tentative grouping of these cases under the title of hereditary purpura. The following are cases of this description:

1. Edward G., 11 years old, suffers from frequent nose bleed, bleeds markedly from cuts, has bled for days following extraction of a tooth, and has numerous "black and blue" marks on his body. He is one of eleven children, ten of whom were boys. The girl is alive and well and normal. Six of the children have died and three of them were bleeders; one died of hemorrhage from the cord when a day old, another when 2½ years old, and a third when 17 years old. Of the four boys who are alive, only this one shows evidence of being a bleeder. The father has given evidences of bleeding inordinately since he was 8 years old; he has suffered from frequent nose bleed all his life, has been in a precarious condition following the extraction of his teeth, and frequently has ecchymoses into the skin. His father and mother died at the age of 75 and 84, respectively, and were never troubled with bleeding. His wife and her parents were normal.

This case was diagnosed as true purpura from the fact that the platelets numbered only 62,500, that the plasma coagulated in eight minutes, and the bleeding time was increased. We may add that this case showed macroplatelets.

2. Morris G., 6½ years old, bled atfer circumcision to such a degree that an operation was necessary. When 2½ years old he fell and bled from the frenum of the lip, requiring the services of a physician. About two years ago a front tooth was pulled and he bled for three or four days and lost considerable blood. Recently he fell and again bled from his lip; after many styptics were applied, the hemorrhage was checked by the physician applying some of his own blood.

There are seven children in this family, five girls and two boys. The only other child who shows a hemorrhagic tendency is the boy, who is 9 years of age, and bled alarmingly when circumcised, and when a tooth was pulled. On this latter occasion he bled for five days and many physicians were called to

see him. He also has frequent nose bleed. The father of the boy gives a history of marked bleeding following circumcision, of frequent nose bleed and of excessive bleeding following cuts. He has a brother and a sister who are well. His wife is perfectly healthy and has three brothers living in this city who do not bleed; her mother and father also had no trouble.

The plasma of this boy coagulated in ten minutes on November 4 and December 2. His platelet count was 98,000 and bleeding time increased.

The following case, which was kindly referred to me by Professor Howell of Baltimore, is striking both in regard to its definite hereditary character and its clear cut purpuric nature. It was observed closely for many months. The following is a summary of the history, which was obtained in detail from Dr. A. E. Fendrich of Weehawken, N. J., who had treated the girl for many years:

Lilian P., 18 years of age, frequent nose bleed and purpuric spots since the age of 12. Menstruation began at 14 and assumed the character of profuse hemorrhage, so that the girl became comatose and was transfused (the mother acting as donor.) Seven months later she developed gangrene of one of her fingers, which had to be amputated. Nose bleeding was frequent and ecchymoses into the skin were present. Somewhat over a year ago there was a hemorrhage into the ankle joint. Menstruation was always profuse and last winter twice threatened life, so that transfusion was performed.

Her father had frequent nose bleed and his family were known as nose bleeders; one of his brothers suffers from nose bleed, one sister died of apoplexy at the age of 45. Her mother always suffered from "blue spots," as did many members of her family; the mother's sister was known to her physician as a "bleeding woman," and has five children who all bleed from the nose frequently; the mother's brother died suddenly, at the age of 35, of heart disease.

Lilian has a brother who bled for two or three days a year ago following the extraction of a tooth; another who has frequent nose bleed, a third who frequently has "black and blue" spots; she has two sisters: one is normal and the other has a nevus extending over her right forearm and hand.

The plasma of this girl coagulated in six to ten minutes in tests made frequently from November to March. Her platelet count was almost always below 100,000, ranging between 80,000 and 90,000. Her bleeding time was increased, her capillary resistance tests markedly positive, her puncture test also positive. This was one of the cases referred to above, where, on occasions, microplatelets were noted, and small, shrunken or fragmented platelets.

This case seems of interest from many aspects. There can be no doubt of the hereditary nature of this purpura. Cases of this description are generally considered hemophilia, and if the heredity points to the father, they may be reported as rare instances of hemophilia inherited from the male. There are one or two other circumstances which should be noted in connection with this history. It will be observed that on one occasion the girl had a hemorrhage into the ankle joint. This is a lesion which is generally regarded as being distinctive of hemophilia. Furthermore, we cannot but be impressed by the num-

ber of disorders in this family which are due, at least in part, to vascular defects; the apoplexy of the aunt at the age of 45; the large nevus of the arm of the sister; the gangrene of the finger of the patient, and perhaps the sudden death of an uncle at the age of 35. As the vessels are probably involved in purpura, these incidents in the family history assume increased significance.[6]

In almost all studies of hemophilia, particular emphasis has been laid on its striking incidence—the fact that it occurs solely in the male and that it is invariably hereditary in origin. Although we have not encountered any case which absolutely controverts these premises, it seems inadvisable and unwarranted to establish such hard and fast limitations as have been set down in the comprehensive monograph on hemophilia recently issued by the Eugenics Laboratory.[7] The literature of the subject cannot be used to decide this question, as it is most difficult to pass on the reported cases, to accept some and to reject others as hemophilia, for conclusive evidence is lacking. For example, there are comparatively few cases cited in this monograph in which a coagulation test of blood, obtained directly from a vessel, has been made, or where a count of the blood platelets is reported. We therefore have to rely solely on the clinical manifestations and, as has been seen from the case (Lillian P.) just reported, these phenomena may be insufficient in themselves to permit of a differential diagnosis. In this connection it should be borne in mind that in other diseases confined to the male sex, diseases which have the advantage of being susceptible to more ready diagnosis, such as color blindness and pseudomuscular dystrophy, well authenticated cases have been described in the female. We raise this question, as we have met with two instances in which a hemorrhagic condition assumed a hemophiliac type in the male, and a purpuric type in the female member of a family. The one case is Yetta K. who is a cousin of Hyman S. and Julius S., whose histories have been recorded above. The coagulation time of the blood of these boys, together with other data, may be seen in Table 2. We may summarize their cases by stating that they have been under observation for some years and that, according to all the diagnostic signs which we have enumerated above, they have classical hemophilia.

6. In purpura there is a tendency to multiple hemorrhages as shown by the great number of petechiae; there is also a hemostatic defect, which is probably associated with the lack of platelets. In hemophilia there is no tendency to frequent hemorrhages, and probably bleeding does not occur more often than normal; there is merely the tendency to excessive bleeding, due to defective blood coagulation.

7. Bulloch and Fildes: Hemophilia, Eugenic Lab. Memoirs, Univ. of London. 1911, xii.

Yetta is their cousin by a maternal aunt, is 18 years of age and well, except for the fact that she suffers from bleeding, which interferes with her work. Since 8 years of age, she has had epistaxis, which has occurred almost daily for the past year. Last year she bled for some weeks following the extraction of a tooth. She also suffers from menorrhagia. Physical examination merely showed that she had numerous ecchymotic marks on her arms and legs. Her plasma coagulated in six minutes; the capillary resistance and puncture tests were markedly positive. The blood platelets were 155,000.

In other words, from a clinical and from a laboratory standpoint, her condition must be diagnosed as purpura rather than hemophilia.

The other instance of this peculiar clinical differentiation according to sex is more interesting, as it involves a sister and brother.

The children were admitted to the Hebrew Infant Asylum last spring. The girl (Millie W.) was almost 4 years old, and the boy 1 year of age. An indefinite and unsatisfactory family history was obtained. Both appeared well when they entered the asylum. Soon afterward, however, both manifested signs of bleeding. The girl bled from the nose and from the bowel and showed a profuse hemorrhagic eruption of the skin, with large ecchymotic spots, as well as numerous pin point hemorrhages, which preponderated on the extremities. The mucous membranes of the mouth and of the vagina, as well as the conjunctivae, were peppered with petechiae. There was oozing from the tip of the tongue. The capillary resistance test was markedly positive; puncture of the skin resulted in a large subcutaneous hemorrhage, the bleeding time was greatly prolonged. The plasma clotted in thirteen minutes on June 25, and in twenty minutes on the following day, and seemed slightly hemolyzed. The platelets numbered 76,000.

The conditions in the boy, who was 1 year old, were quite different. He gave a history of having bled for ten days following circumcision. He was somewhat pale, although not markedly anemic looking. Hemoglobin 63 per cent.; red blood cells, 4,800,000; weight, 17½ pounds. The appearance of the hemorrhagic skin lesions was totally different from that of the girl.

There were only two subcutaneous hemorrhages, an ecchymosis over the knee and another over the forehead, and an absence of small petechiae. The bleeding time was slightly prolonged, the puncture test and the capillary resistance test absolutely negative. The blood platelets numbered 351,000 on one occasion, 400,000 on another. The plasma coagulated once in twenty-one minutes and in thirty minutes on the following day. There was no bleeding from the bowels. Pain, and possibly hemorrhage, was present in the knee joint.

Here then are two contrasting pictures. The girl with purpura typical in all respects, corresponding in its various clinical and laboratory aspects to the description which has been sketched above; the boy showing a quite different symptomatology, corresponding to hemophilia. The basis of this difference is entirely obscure, although it is possible to suggest attractive hypotheses. Similar cases should be looked for in the future, and the possible bearing of sex on the manifestations of other diseases, particularly blood diseases, should be borne in mind.

Although it is advisable to regard true hemophilia as a clearly defined entity, and to sharply contrast it with purpura, both of these

disorders probably embrace various types of disease. Recently we described a case[8] of hemophilia which had been under observation for a long period and was exceptional in having a deficiency of calcium in

TABLE 3.—COAGULATION TIME OF CALCIPRIVA CASE

Date	Coagulation Time Plasma minutes	Coagulation Time Blood minutes	Remarks
1914			
April 21....	58	29	Control plasma clotted in eight minutes; capillary resistance test negative.
May 21.....	16	11½	Frontal hematoma, nose bleed. Platelets 487,000. Antithrombin normal in first test, increased in this test compared with controls.
May 23.....	30	.....	Platelets 367,000.
May 26.....	28	.....	
May 28.....	28	.....	
June 15.....	22	.....	Has many "black and blue" marks.
June 25.....	18	.....	
Aug. 19.....	12	10	*Has been bleeding* from finger for some days. The addition of calcium did not hasten coagulation of the blood. The plasma clotted in optimal time in the tube with only two drops of calcium solution added.
Aug. 24.....	12	.....	Antithrombin not increased. Has hemarthrosis of hip.
Oct. 24.....	.....	15	Blood clotted in eight minutes on addition of Ca.
.....	.....	15	Blood clotted in eight minutes on addition of Ca., whereas control blood was delayed. Has had nose bleed twice two days ago.
Nov. 10.....	32	15	Has received calcium lactate for four days. The addition of calcium did not hasten coagulation.
Nov. 17.....	34	.....	
Dec. 2.....	12	7	*Has been receiving calcium lactate for some weeks.* Calcium delayed clotting time when added to the blood. Has not bled for some time. Looks well.
Dec. 28.....	34	12	
Dec. 29.....	.....	40	Calcium did not hasten coagulation.
1915			
June 26.....	88	.....	
July 2.....	60	.....	
July 9.....	.....	34	Addition of calcium made clotting time thirteen minutes.
Sept. 8.....	22	.....	

the blood. As is well known, although in the early studies of hemophilia attention was directed to the calcium factor, it was shown by

8. Hess, A. F.: Bull. Johns Hopkins Hosp., November, 1915.

Nolf and others that calcium was present in normal amount in the blood, and by Morawitz and Lossen that an addition of lime salt does not hasten coagulation. We shall not repeat the details of this case which, as far as we know, is unique, but have summarized some of the data in Tables 3 and 4, and may add that the addition of a small

TABLE 4.—Effect on Clotting Time of Addition of Calcium Solution to Blood

Clotting Time minutes	Control	Drops of Ca Solution Added			
		1	2	3	4
A. NORMAL CASE					
4	+	.....	+	+	+
5	+ +	.....	+ +	+ +	+
6	+ + + *	.....	+ + +	+ +	+ +
7	.....	.....	.....	+ +	+ +
9	.....	.....	.....	+ +	+ +
11	.....	.....	.....	+ +	+ +
13	.....	.....	.....	+ +	+ +
15	.....	.....	.....	+ + +	+ +
17	.....	.....	.....	.....	+ +
B. HEMOPHILIA CALCIPRIVA					
5	—	—	—	—	—
9	—	—	—	—	—
11	—	+	+ +	—	—
13	—	+	+ + +	+	—
17	—	+ + +	.....	+ +	+
21	—	.....	.....	+ +	+ +
26	—	.....	.....	+ + +	+ + +
34	+ + +	.....	.....	.....	.....
C. TYPICAL HEMOPHILIA					
4	—	—	—	—	—
8	—	—	—	—	—
10	+	+ +	+	+ +	+
14	+ +	+ +	+ +	+ +	+ +
18	+ + +	+ +	+ +	+ +	+ +
22	.....	+ +	+ +	+ +	+ +
26	.....	+ +	+ + +	+ +	+ +
30	.....	.....	.....	+ + +	+ +

* + + + denotes complete clotting.

amount of a weak calcium solution did hasten coagulation; that a subnormal amount of calcium was found by analysis in the blood; that a metabolism test showed a negative balance for calcium, which became positive when lime was added to the diet, whereas a control metabolism test of a typical hemophiliac (Julius S.) was normal in all respects.

These chemical tests were carried out by Dr. Max Kahn. We have here then an atypical hemophilia, a hemophilia calcipriva. Probably similar cases will be found, and other instances of hemophilia differing in one factor or another from the classical model, will be encountered from time to time.

Purpura is far less well defined than hemophilia. From a clinical point of view, it would seem advisable for the present to have purpura include all bleeders who show a deficiency of blood platelets. These cases will manifest many or most of the signs which have been discussed at the beginning of this paper. It is realized that a grouping which rests on such a basis may have no unity from an etiologic standpoint and merely be tentative in nature. It furnishes, however, that which does not exist at present, a definite criterion, namely, platelet deficiency, just as retarded coagulation of the blood is regarded as the essential diagostic factor in hemophilia. Most cases of purpura conform to this standard. Not all, however. There are cases which cannot be classified because they manifest the characteristic signs of both purpura and hemophilia. To illustrate:

Jessie K., a girl of 4 years, seen with Dr. Cleaver, had large subcutaneous hemorrhages and bleeding from the bowel. The condition could not be diagnosed; there was in addition an enlarged spleen and liver and the history of fever some months previously. The points of interest were that the platelet count was but 21,000, and the plasma did not clot at all on the addition of calcium, at least not sufficiently to allow the vial to be turned over. The whole blood also did not clot solidly. There was no excess of antithrombin, and the addition of calcium and of fibrinogen did not hasten coagulation in vitro. Fibrinolysis was present.

If we exclude cases showing markedly delayed coagulation time from the group of purpuras, this case cannot be considered purpura; if at the same time we consider a deficiency of platelets as incompatible with hemophilia, the case must rest unclassified. Although, as Howell states in the article referred to above, "the main distinguishing feature of the hemophilic condition is the greatly delayed coagulation time of the blood," it is seen that other diseases can bring about this disturbance of clotting to the same degree.

When we turn to a consideration of the rôle of the blood vessels in the hemorrhagic diseases, we begin to tread on still more uncertain ground. Very little is known of this factor in hemorrhage, and yet it is clear that it not only plays a part, but must play an important part. The capillary resistance test must depend on an abnormal permeability or weakness of the vessel wall, and its almost regular occurrence in purpura necessitates the deduction that the vessels are involved in this disorder. In the course of our study we have encountered, from time to time, cases in which hemorrhage occurred which seemed to be the result of vascular weakness. This lack of stability of the vessel wall

would seem to be a congenital defect, if we may judge from its manifestation in early life. The following cases can best be explained on the basis of a weakness or fragility of the vessel walls:

Bessie B., a child 3 years old, developed pertussis last year at the time of an epidemic which attacked about eighty children. She had a moderately severe case. Nevertheless she was the only child who developed subconjunctival hemorrhages. These effusions showed themselves from time to time in the course of the disease, following a paroxysm of coughing. The coagulation time of the plasma was normal; platelets also normal, 324,000; capillary resistance test positive, v. Pirquet positive.[9]

We attribute the hemorrhages in this case to the additional strain put on weak vessels at the time of the paroxysmal cough. In a measure this results in a spontaneous or automatic capillary resistance test. The case which we next cite is to be interpreted in the same way:

At the Willard Parker Hospital there are about twenty children who have to wear permanent intubation tubes on account of laryngeal obstruction. These children are frequently intubated. One of this unfortunate group always develops petechiae of the face and neck following the insertion of the tubes, which causes some struggling and marked venous congestion. The plasma of this child clots in the normal time; the platelets are not deficient. This is the only child who manifests this phenomenon.

As is well known, many of the infectious diseases lead to a rash, which is more or less hemorrhagic. Scarlet fever may be cited as a notable example of a disease in which the exanthema is petechial. A few petechiae are frequently present also in measles. We have had an opportunity of testing numerous cases of scarlet fever by the capillary resistance test and have found it in most cases positive, becoming negative again during convalescence. Although we do not care to agitate the interesting but highly hypothetical topic of vascular toxins, we may venture the opinion that these petechiae are due to a temporary lesion of the blood vessel wall. The fact that the petechial rash is not always in direct relation to the severity of the disease would suggest an individual susceptibility of the vessels. Here again the blood platelets were found normal and the coagulation of the plasma not delayed. (Table 5.)

The blood vessels may be abnormally weak and, as we have shown, yield to an undue pressure as in pertussis, they may be rendered permeable as the result of the infectious disease, or a similar result may be brought about by nutritional disturbances. This phase of the problem was studied recently in connection with infantile scurvy and

9. There seems to be some significance in the fact that of the seven children in the institution which we have been observing on account of a tendency to develop "black and blue" marks, four give a positive tuberculin reaction. Their blood coagulates in normal time. The ecchymoses appear especially in the spring, the season when tuberculous meningitis and phlyctenulae occur most often.

reported on elsewhere.[8] This investigation led to the conclusion that the hemorrhagic manifestations of this disorder—the subperiosteal bleeding, the cutaneous petechiae, the red cells in the urine—cannot be accounted for by disturbances of the blood itself. The blood coagulates in normal or almost normal time, there is no increase in antithrombin nor a decrease in the platelets. On the other hand, the puncture test and the capillary resistance test are almost always positive. This involvement of the vessel wall may not be elective, but merely part of the general tissue change in this disorder. We have incorporated

TABLE 5.—Coagulation Tests of Plasma

Type of Case	Drops of Calcium Chlorid (0.5 Per Cent.)	Coag. Time (5 Drops of Oxalated Plasma) minutes
Infantile scurvy	1	12
	2	10
	3	9
	4	9
	5	9
Child showing frequent "black and blue" marks	1	6
	2	6
	3	6
	4	6
	5	8
Hemorrhagic measles	1	7
	2	6
	3	6
	4	6
	5	6
Normal	1	7
	2	6
	3	6
	4	6
	5	7

in a table (Table 5) coagulation tests of a case of infantile scurvy, of hemorrhagic measles, and of plasma from a child with a tendency to nose bleed and ecchymoses. It will be noted that the plasma coagulated in normal time, that of the scurvy case being slightly delayed.

From time to time in the course of this paper, tests for antithrombin have been referred to. It will be well, therefore, before concluding, to say a word about this substance and its estimation. Some years ago, Weil, of Lyons, who was the first to venture to carry out venepuncture in hemophilia, suggested that the retarded coagulability in this disorder was due to an excess of antithrombin. Since then it has

been demonstrated, however, that such is not the case. The tests in Table 6, which show the effect of the addition of normal and of hemophiliac antithrombin to normal blood, are a convincing illustration of this fact. It will be noted in this table that a specimen of blood which clotted in five minutes, acquired a coagulation time of twenty-one minutes when one drop of antithrombin was added, irrespective of whether the antithrombin was from a normal person or from a case of hemophilia. This was likewise true in another specimen of normal blood (Dr. B.)[10] which was similarly tested. For tests of plasma antithrombin the method of Howell was employed, which consists of rendering inactive the prothrombin in plasma by means of a degree of heat which at the same time will not destroy the antithrombin. A fibrinogin solution is added to this heated and filtered plasma, and then the number

TABLE 6.—Effect of Adding Antithrombin of Normal and Hemophilic Individuals to Normal Blood

Substance Added to Blood	Normal Blood I (Dr. B.) Coagulation Time (10 Drops) minutes	Normal Blood II (Dr. R.) Coagulation Time (10 Drops) minutes
.....	5	
One drop Ca (0.5 per cent.).....	10	6½
Two drops fibrinogen.....	10	7
One drop *normal* antithrombin.....	21	30
Two drops *normal* antithrombin.....	*	*
One drop *hemophilic* antithrombin.....	21	30
Two drops *hemophilic* antithrombin.....	*	*

* No clot.

of drops of thrombin is determined which it is necessary to add to bring rapid coagulation. A test of this kind is shown in Table 7. We see a comparison of the blood of a child with a "bleeding tendency," of a normal individual, and of the atypical hemophilia (calcipriva) mentioned above. This test shows that the absolute amount of antithrombin in this case of hemophilia was at times in excess. It varied considerably from time to time, suggesting that there may be a peculiar interrelationship between calcium and antithrombin. In addition to the Howell test, we made use of an antithrombin test described by me,[11] which has the advantage of being simple, as normal plasma is employed instead of solutions of fibrinogen and of thrombin.

10. This table also shows that the addition of a solution of calcium or of fibrinogen retards rather than hastens the coagulation of normal blood.

11. Hess, A. F.: Jour. Exper. Med., 1915, xxi, 338.

CONCLUSIONS

The main points in the preceding article may be summarized as follows: The coagulation time of the plasma in hemophilia at times may become normal without the occurrence of hemorrhage or other apparent change in the condition of the patient.

The estimation of the number of blood platelets is of great value, as has been found by others, in differentiating between purpura and hemophilia. In some cases of purpura, the platelets are abnormal and may be differentiated, like other macrocytes and microcytes of the blood, into *macroplatelets and microplatelets*.

TABLE 7.—ANTITHROMBIN TESTS OF PLASMA

Type of Case	Thrombin in Drops	Coagulation Time minutes
Hemorrhagic tendency	1	6
	2	4
	3	4
	4	2
Normal	1	18
	2	6
	3	4
	4	2
Hemophilia calcipriva	1	66
	2	16
	3	7
	4	5

The puncture test—the reaction following subcutaneous puncture of the skin—is an aid to diagnosis. In hemophilia a hemorrhagic area rarely results from this procedure; in purpura it is the rule.

The capillary resistance test is also of value. By this is understood the reaction following the application for a definite period of a tourniquet to the upper arm. In purpura, this results in petechial hemorrhages on the forearm; in hemophilia the effect is negative.

There is an *hereditary purpura* as well as hereditary hemophilia. This type of purpura should be more generally recognized, so that these cases will not, on account of their hereditary history, continue to be regarded as hemophilia.

The male member of a family may be a bleeder of the hemophiliac type and the female of the purpuric type. Two families are described in which one member suffered from hemophilia and another from purpura.

Hemophilia may be atypical: A case is reported which showed a calcium deficiency, as borne out by various chemical and clinical tests (hemophilia calcipriva).

In some cases manifesting hemorrhage, the vessels seem to be involved. This weakness is encountered in children and may be congenital; it may appear in the course of an infectious disease, or of a nutritional disorder, such as infantile scurvy.

In the classical case the differentiation between hemophilia and purpura is simple. The picture of a typical hemophiliac is a male, with a hereditary history of bleeding, whose blood manifests a definite delay in coagulation time, whose platelets are normal in number, "bleeding-time" not increased, who shows no hemorrhagic reaction following subcutaneous puncture of the skin, and a negative capillary resistance test. A typical case of purpura is found to be quite different: the patient may be a male or a female, the plasma coagulates in almost normal time and the number of blood platelets is decreased (frequently below 100,000 in number), there is definite subcutaneous hemorrhage following puncture of the skin, an increase of the bleeding time, and the development of a large number of petechial hemorrhages following the application of a tourniquet.

16 West Eighty-Sixth Street.

EVENTRATION OF THE DIAPHRAGM

WITH REPORT OF A CASE OF RIGHT-SIDED EVENTRATION *

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Among the lesions of the diaphragm, none has passed into the literature under more synonyms than the condition commonly called *"eventration."* Since 1849, when Cruveilhier introduced his conception of the disease under this term, the names: "dilatation," "relaxation" (Wieting), "insufficiency" (Franck), "high position," "elevation" (Griffin), have been used to designate a pathological state of the diaphragm, characterized by a general expansion of one half of the organ, allowing the abdominal viscera to be displaced upward into the thoracic cavity. The diaphragm is greatly thinned as well as distended, but its three layers remain intact, and there is no solution of its continuity. In this essential respect, the condition is different from hernia of the diaphragm, which, whether true or false, depending on the presence or absence of a hernial sac, consists of a localized opening in the sheet of the diaphragm, through which the abdominal viscera pass into the thoracic cavity. All of the terms mentioned are partially descriptive, though none is satisfactory. While the commonest of them, "eventration," is a gross misnomer, since it suggests the displacement of the viscera out of the abdomen, it has received by custom a connotation which is specific for this condition of the diaphragm. The other terms are so ambiguous that if any one of them were accepted, it would yet have to acquire a long usage to gain the special meaning needed in this connection. As its multiplicity of names suggests, eventration is one of the rare lesions of the diaphragm. The latest statistics of its frequency as compared with other types of hernia of the diaphragm, published by Eppinger in 1911, show the following incidence:

TABLE 1.—FREQUENCY OF HERNIA AND EVENTRATION

Type of Diaphragmatic Hernia	Right Side	Left Side
Hernia vera	21	53
Hernia spuria	34	527
Eventratio	2	15

Total cases of hernia, 635. Total cases of eventration, 17. Ratio of eventration to hernia, 1 to 37.

* Submitted for publication Oct. 11, 1915.

* From the Medical Clinic of The Johns Hopkins Hospital.

Since the compilation of these figures, many cases of hernia have been reported, and the number of cases of eventration has risen to forty-five. But the relative incidence of these lesions has probably remained so constant that this proportion is still a fair index of the rarity of the condition. This table is further interesting in showing the relative infrequency of hernia and eventration of the right side of the diaphragm. The case to be reported in this paper is thus seen to be the third example of right-sided eventration.

Thirty-one cases of eventration of the diaphragm were collected and summarized by Bergmann in 1913. A review of the literature, however, reveals several reports omitted by him and, in addition, several published since his article. The following is a tabulation of forty-five case-reports—forty-four gathered from the literature and one new case, reported in this paper.

REPORT OF CASE

Diagnosis.—Eventration of the right side of the diaphragm. Congenital malformation of the mesentery, with mesenteric hernia. Chronic intestinal obstruction.

History.—J. B., a white male, aged 52, was admitted to the Medical Clinic of The Johns Hopkins Hospital, November 16, 1914. General No. 99055. Complaint, "Pain in the upper right side of the stomach."

The patient came from healthy stock and was the father of five normal children. In general his health had been robust, except for typhoid fever, complicated by pneumonia, when he was 19 years old, he had not been incapacitated by any acute infectious disease. In his occupation as a sailor, chiefly around harbors, he had led an active life. He was not addicted to the use of alcohol or tobacco.

Throughout his life a "sensitiveness of the stomach" had necessitated a moderate degree of care in the selection of his diet. In 1909, five years ago, he began to have "indigestion," associated with constipation and pains in the upper right quadrant of the abdomen. This pain was "knife-like," extending from the midline to the axilla, above the umbilicus and below the costal margin. The pain could be relieved by pressure on that side of the abdomen. The pain recurred at irregular intervals, and sometimes was accompanied by nausea and vomiting. He was never jaundiced and did not have tarry stools. He had not been dyspneic. Lately the pain has recurred more frequently and has radiated into the right shoulder. This has incapacitated the patient, and he has lost 17 pounds in the past five months.

Physical Examination.—The patient, a large-framed, well nourished man, is neither dyspneic, cyanotic, nor jaundiced. The general examination is unimportant, except as regards the thorax and abdomen.

Thorax: The thorax is deep, with a wide costal angle. Both sides expand equally; by measurement, the right from 45 to 47 cm., the left from 45 to 46 cm. On the left side there are no abnormal signs. On the *right side*, vocal fremitus is not palpable in the axilla. The percussion note at three successive levels changes from resonance to flatness and to tympany. The note is resonant over the upper right front and back. At the third rib it becomes dull, and below this it is flat to the level of the fifth rib. From the fifth rib downward the note has a dull tympanitic quality, until the full abdominal tympany is reached. At the base posteriorly the lower border of pulmonary resonance is 10 cm. higher on the right than on the left, and only slight descent of this is percepti-

ble on the right. A tympanitic area occupies the usually dull region of the lower ribs in the back. Litten's sign is absent in the right axilla. On auscultation, enfeebled breath sounds are heard over the upper portions of the right lung. These sounds gradually diminish until below the level of the fourth rib the sounds of breathing and of voice are inaudible. There is no egophony.

Abdomen: The abdomen is not distended, but on the right side the muscles are resistant. Varying irregular masses are palpable in the right upper quadrant, in a region which is painful when pressed on. The edge of the liver cannot be felt, and the area of hepatic dulness seems to end at the level of the fifth rib. In the region of the lower ribs behind, the percussion note is tympanitic, as it is over the front of the right upper quadrant. On the left side there is a small inguinal hernia. *Inflation of the colon,* by pumping air into the rectum, causes the abdomen to become distended, while the level of tympany on the right side is advanced upward an interspace. This distention causes pain like the distress which characterizes the patient's illness.

Results of Special Examinations.—Gastric Analysis: Free HCl, 34; total acidity, 72.

Feces normal. Urine normal.

Wassermann reaction negative. R. B. C., 4,792,000; Hb., 72 per cent.; W. B. C., 7,440. Widal test negative. Conjunctival tuberculin test, 5 per cent., slightly positive.

Heart: The heart is not enlarged or displaced. The electrocardiogram (Film H-09) shows "normal rhythm, with no abnormal complexes. Conduction time 0.18 second. There is a progressive decrease in the size of the R-wave. Normal electrocardiogram."

As the result of these examinations, Dr. Janeway excluded thickened pleura, which among other conditions had been considered the likely explanation of the abnormal physical signs over the right side of the chest, and made the diagnosis of eventration of the right side of the diaphragm. This conception was strengthened by the following radiographic studies, and when confirmed at subsequent operation, proved to be the first instance of the *intra vitam* diagnosis of this condition.

Roentgenologic Findings (Dr. Baetjer and Dr. Waters):

Thorax: (Plate 26278. Fig. 1). The lung shadows are normal. The dome of the right diaphragm is very high, reaching approximately the level of the third rib in front. It is normal in shape.

The *fluoroscopic* examination shows on the right side a high diaphragm, which is normal in shape. On deep respiration the movement of this side is paradoxical; it moves upward during inspiration and downward during expiration. The left side of the diaphragm moves freely in long sweeps downward during inspiration. The heart appears to be normally placed. The stomach is in a high position with its pylorus under the right costal margin. The colon, filled with bismuth, shows double and complicated kinking on the right side.

Large Intestine: (Plate 26196. Fig. 2). This shows the descending colon and the splenic flexure in their normal situations. The transverse colon is prolapsed, and in the right upper quadrant it is looped and doubled on itself. On the right side of the plate there is no shadow to indicate the position of the ascending colon.

These evidences of abdominal disease confirmed the impression that a chronic inflammatory condition, possibly cholecystitis, was present in the right upper quadrant. Because of this, an exploratory laparotomy was performed by Dr. J. M. T. Finney, Dec. 4, 1914. The following is the summary of the note of operation (see Fig. 3):

Neither the liver nor the gallbladder is visible through the right rectus incision. The gallbladder is found roughly a hand's breadth above the costal margin, at the level of the sixth rib. It is normal. The liver, slanting downward and to the left, looks normal, but is situated high up under the ribs.

TABLE 2.—SUMMARY OF CASES—

Case No.	Year	Author	Sex*	Age	Side Affected	Chief Clinical Findings
1	1784	Pyl.....	♂	1 da.	Left	Death soon after birth. Cyanosis.....
2	1790	Petit.....	♂	ad.	Left	"Asthma, after eating".....
3	1819	Meckel.....	♂	fet.	Left	.....
4	1829	Laennec.....	♂	ad.	Left	.....
5	1837	Froriep.....	♀	19	Left	Dyspnea and digestive disorders for 14 years...
6	1852	Lawrence.....	♂	33	Left	Practically no symptoms.....
7	1867	Marsh.....	♂	33	Left	No history .....
8	1882	Thoma.....	♂	75	Left	No history .....
9	1894	Tennant.....	♂	60	Left	No history .....
10	1900	Crispino.....	♀	55	Left	Gastro-intestinal disorders. Fractured arm....
11	1901	Struppler.....	♂	26	Left	Life-long cough. Dyspnea after trauma. Physical signs of hernia. Roentgen-ray signs of eventration
12	1901	Neisser.....	♂	42	Left	Bronchiectasis. Signs of intestines in left chest. Roentgen ray of eventration
13	1902	Doering.....	♂	60	Left	Dyspnea many years. Signs of hernia.....
14	1902	Fraenkel.....	♂	40	Left	Dyspnea and hemoptysis several years. Signs of hernia by phys. exam. and Roentgen ray
15	1908 1905	Glaser Hildebrand and Hess	♂	39	Left	Incarceration symptoms for many years. Famous case of "Sch."
16	1905	Saller and Rhein	♂	30	Left	Signs of subdiaphragmatic gas abscess.....
17	1906	Wieting.....	♂	50	Right	Symptoms of gallstones. Operation.....
18	1907	Kienböck.....	♂	54	Left	Trauma to right side years previously. Signs of hernia. Roentgen ray of eventration
19	1907	Hers.....	♂	36	Left	Cough, Typical signs of eventration.....
20	1908	Arnsperger.....	♀	20	Left	No symptoms. All signs of eventration.....
21	1909	Königer.....	♀	27	Left	Dyspnea and pain several years. The usual signs of eventration
22	1910	Otten and Schefold	♂	68	Left	Paradoxical dysphagia. Usual signs.....
23	1910	Beltz.....	♂	70	Left	Dyspnea, pain in left side, gastric disorders. Typical findings
24	1910	Beltz.....	♀	34	Left	No history .....
25	1911	Scholz.....	♂	1	Left	Slight dyspnea and indigestion.....
26	1911	Eppinger.....	♂	35	Right	Typical signs of eventration. Case not seen in life
27	1911	Franck.....	♂	73	Left	Right hemiplegia 12 years. Chronic constipation. Typical eventration
28	1911	Appel.....	♂	60	Left	Dyspnea 2 years. Typical eventration.....
29	1911	Becker.....	♂	25	Left	Life-long gastric discomfort. Typical.....
30	1912	Scheidemandel..	♂	71	Left	Pain between shoulders. Usual signs.....
31	1912	Scheidemandel..	♂	55	Left	Cough 26 years. Typical signs.....
32	1912	Malkow.....	♂	48	Left	Typical signs, according to Bergmann.....
33	1913	Bergmann.....	♂	60	Left	Gradual onset of dyspnea. Typical.....
34	1913	Kayser.....	♀	28	Left	No symptoms. Typical findings.....
35	1913	Haase.....	child	3 mos.	Left	Cough and dyspnea. Typical findings.....
36	1913	Reuss.....	♂	46	Left	Injured left side. Typical findings.....
37	1913	Baetge.....	♂	64	Left	Dyspnea and palpitation many years. Typical signs of eventration
38	1913	Baetge.....	♂	28	Left	Gastro-intestinal disorders. Typical eventration
39	1913	Baetge.....	♂	41	Left	Dyspnea and stomach trouble since childhood. Typical signs
40	1913	Motzfeldt.....	♀	41	Left	Practically same as Case 39.....
41	1913	Krause.....	♂	42	Left	Gastric disorders. Typical signs.....
42	1913	Krause.....	♀	36	Left	After pregnancy. Typical signs.....
43	1914	Manges and Wessler	♂	35	Left	Gastric crises, dyspnea, cyanosis. Typical signs of eventration
44	1914	Fischer.....	♂	30	Left	Found at conclusion of typhoid fever complicated by pneumonia
45	1915	Author's case...	♂	52	Right	Painful febrile periods suggesting gallbladder disease. Tympany over lower right chest. Right diaphragm by Roentgen ray at third rib

* ♂ = male; ♀ = female.

Associated Anomalies	Probable Etiology	Cause of Death	Condition of Diaphragm—Necropsy	Remarks
None.....	Congenital	.....	Whole side a thin membranous sac	
None.....	Acquired?	Peritonitis	Whole side thin and distended.....	Cruveilhier's case
None.....	Congenital	.....	One half diaphragm a thin sac	
None	.....	.....	Same as above	
None.....	Congenital	.....	Thin distention of whole side, with three layers intact	
None.....	Congenital	Pneumonia	Thin aponeurotic expansion of entire left half of diaphragm	
None.....	Congenital	Pneumonia	Same as above	
Inguinal femoral and pelvic herniae	Congenital	Peritonitis	Pleura, peritoneum and a little fibrous tissue representing the diaphragm	
None.....	Congenital	Pneumonia	Thin, distended sac	
Dextrocardia..	Congenital	?	Left diaphragm some inches higher than the right	
None.....	Acquired?	.....	.....	Shown by Arnsperger to be eventr.
None.....	Congenital	.....	.....	Atrophy of lung
3 lobed left lung	Congenital	.....	Thin membranous sac of whole side with usual layers	Lung not compressed
None.....	Congenital	Cancer of tongue	Thin sac, showing "lipomatous pseudohypertrophy"	Benda's case
Small left lobe liver	Congenital	Peritonitis	Thin sac formed by whole of left side of diaphragm (Eggeling)	Intragastric pressure
Small left lung	Congenital	Typhoid fever	Thin sac, with few muscle fibers	
None.....	Congenital	Pyonephrosis	Thin sac with liver and stomach, ruptured by perforated gastric ulcer	First case of right sided eventration
None.....	Acquired			
None.....	Congenital			
None.....	Congenital			
None.....	Congenital			
None.....	Congenital			
None.....	Acquired...	.....	.....	Example of chronic "magenblase"
None.....	Acquired?..	Pneumonia	Saccular distention of whole side	Atrophy of phrenic nerve
None	Congenital			
None.....	Congenital	.....	Diffuse expansion of right side	
None.....	Congenital	.....	.....	Paradoxical movement
None.....	Congenital			
None.....	Congenital			
None.....	Congenital			
None.....	Congenital	.....	.....	Diphtheria at 20
None.....	Congenital			
None.....	Congenital	.....	.....	Syphilis
None.....	Congenital	.....	.....	Purpura
None.....	Congenital	.....	.....	Pneumonia
None.....	Acquired?..	.....	.....	Jamin's sign
None.....	Congenital			
None.....	Congenital	.....	Relaxed, elevated diaphragm found at operation for gallstone	Electrocardiogram. Cardiorenal
None.....	Congenital			
Stunted left lung. Dextrocardia	Congenital	Nephritis..	Atrophy and fatty change of distended left half of diaphragm	Anomaly of mesenteric arteries
None.....	Congenital	?	Sac-like left diaphragm.....	Phrenic atrophied
None.....	Acquired			
None.....	Acquired?			
None.....	Acquired?..	.....	Thin, dilated diaphragm, found at operation	
Malformation of mesentery	Congenital	Pulmonary embolism	Thin distention of whole right side of diaphragm reaching level of R. 2; found at operation	Paradoxical movement

The hand passed over the dome of the liver touches the third rib in front, and by slight pressure the second interspace can be reached. The diaphragm is thin, but otherwise normal. It can be felt to cover the liver smoothly, and has no localized bulging or solution of continuity.

The stomach is normal, but has its pyloric end high under the right costal margin. The transverse colon is redundant and together with a loop of small intestine passes through a hernia in the root of the mesentery. In this anomalous formation of the mesentery there are large dilated veins and an aberrant artery. The cecum is high under the costal margin on the right side.

From these notes and from a sketch made at the operation, Mr. Brödel elaborated the drawing reproduced as Figure 3. This shows clearly the anatomical relationships of the high diaphragm and the displaced viscera, and at a glance, offers the explanation of both the gastro-intestinal symptoms and also the physical signs over the chest and abdomen.

The patient progressed well for ten days after the operation, when suddenly he became dyspneic, spat blood, and died after a few minutes, with symptoms of pulmonary embolism. As a necropsy was not permitted, no further examination of the diaphragm was possible.

DIFFERENTIAL DIAGNOSIS

Previous discussions of the differential diagnosis between eventration and conditions presenting similar signs have been limited to the consideration of left-sided lesions. The case just described directs emphasis on the diseases of the right thoracico-abdominal section of the body. At the same time, however, completeness and clarity require a review of the findings in the left-sided cases.

In the case described herein, the abdominal symptoms were the urgent cause of the patient's coming to the hospital. The intermittent periods of pain in the right upper quadrant, associated with vomiting and fever, made it seem likely that there was some chronic inflammatory disease in that region. Among the diagnoses considered were duodenal ulcer, with perforation and a localized gas abscess beneath the right lobe of the liver, kinking of the transverse colon, producing intestinal obstruction, and cholecystitis. This last diagnosis was considered the most probable, and was the point determining the operation on the patient. The mesenteric hernia, found at operation, must have produced frequent mild incarcerations of the intestines passing through it. This explanation is sufficient to permit the dismissal here of this phase of the subject.

In the absence of signs of fluid, thickened pleura over the lower part of the right chest was thought at first to be the cause of the dulness on percussion, the diminution of the breath- and voice-sounds, the absence of fremitus and of Litten's sign in the right axilla. However, the exact location of the flatness and of the complete loss of vocal sounds over a region from the third to the fifth ribs, lying between normal lung resonance above and a curious area of tympany below, made it seem more likely that this region was occupied by the liver. Inflation of the colon displaced this area of dulness upward

and increased the tympanitic region below, as would occur with a semimovable organ like the liver lying in the relaxed and elevated dome of the diaphragm, but which would not occur in thickened pleura. The most closely allied condition, which would give some of these signs, is hernia of the right side of the diaphragm. Of this lesion, for comparison, we have only one case—perhaps the only case diagnosed during the life of the patient—namely, that reported by Dietlen and Knierim. In their case, the stomach and some coils of intestine passing through the diaphragm into the thoracic cavity, lay above the liver. Here, the area of tympany across the chest, lay between normal lung resonance and the area of hepatic dulness. Changes in the position of the patient and the ingestion of fluids and gases somewhat modified the sounds elicited by percussion and auscultation over the tympanitic area, giving almost the reverse order of the two lower strata of physical signs noted in our case of eventration of the diaphragm.

In our case, the radiographic and fluoroscopic examinations confirmed the clinical impression of eventration of the diaphragm. The left side appeared to be normal, while the right showed a smooth shadow arching upward across the thorax to the level of the third rib (Fig. 1). Above this shadow, normal pulmonary shadows were visible, while below it, the stomach and intestines, when filled with bismuth, could be clearly seen. This contrasts sharply with the roentgenograms in hernia of the right side of the diaphragm. In Dietlen's and Kneirim's case, a bright area was seen between the lung shadows and the black hepatic region. When bismuth paste was administered, the peristaltic shadows of the stomach and small intestine obliterated this bright idea (Fig. 4). The movement of the diaphragm in these two cases is so contradictory that it indicates the doubtful value of this sign in the differentiation of eventration from hernia of either the right or the left side of the diaphragm. In 1898, Kienböck observed paradoxical movement of the diaphragm by the fluoroscopic screen. This form of motion is characterized by inspiratory elevation and expiratory descent of the affected side of the diaphragm. Though it is found in numerous conditions, such as pleurisy with effusion, and pyopneumothorax, it was observed in a case of hernia of the left side of the diaphragm, and seized on as one of the definite radioscopic signs of this condition. In our case of eventration, the movement of the thin distended side of the diaphragm was paradoxical in character, while in the case of hernia, the movement of the upper borders of the diaphragmatic shadows was normal, descending with inspiration and rising with expiration. Before this, however, Lotze's case had proved that the differential diagnosis between hernia and eventration cannot

be made with absolute certainty by means of the Roentgen ray. He described what seemed to be a typical example of eventration of the left side of the diaphragm, in which the respiratory excursions of the diaphragm were normal, though diminished. Several years later, when Risel performed the autopsy on this patient, he found an old hernia of the left side of the diaphragm, with the rest of the organ practically normal.

A most decisive differential fact may be gained from the study of the intragastric pressure, according to the method of Marey and

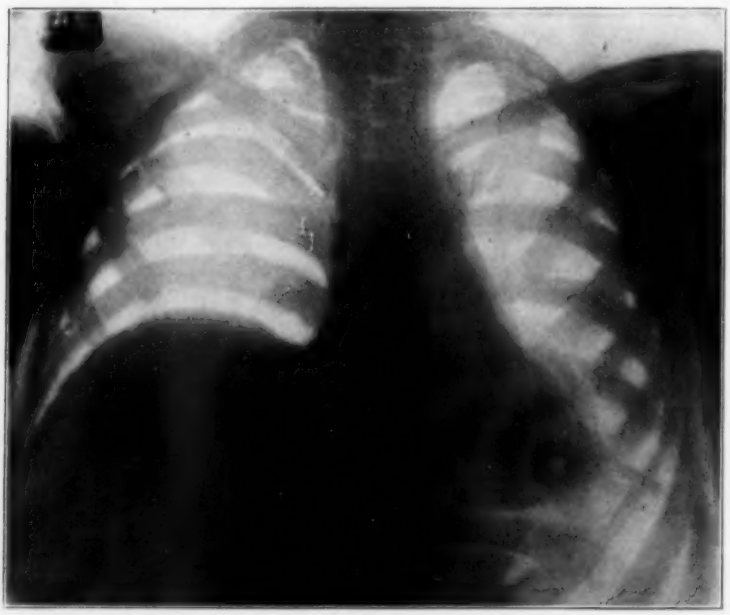

Fig. 1 (Roentgen-Ray Plate 26278).—Thorax: showing the dome of the left diaphragm, normal in outline, arching to the level of the third rib in front. Above this are normal lung shadows, and below the hepatic shadow is a bright area occupied by the intestines displaced upward. On the left the heart and diaphragm are in normal position.

Schlippe. By the application of this method, Hildebrand and Hess correctly diagnosed as eventration of the left side of the diaphragm, the famous case of "Sch.," which, for a period of twelve years, was studied in many clinics as an example of hernia. By suitable instruments, a record is made of the respiratory phases of the pressure within the stomach, to determine whether the organ is situated within the abdominal or thoracic cavity. If the stomach is in its usual place,

the pressure within it rises during inspiration and falls during expiration, synchronously with the intra-abdominal pressure. If, however, the stomach lies in the thoracic cavity, the curves are reversed; the pressure falls during inspiration and rises during expiration, synchronously with the changes in the intrathoracic pressure. This investigation was not made in our case, but Dietlen and Knierim found that

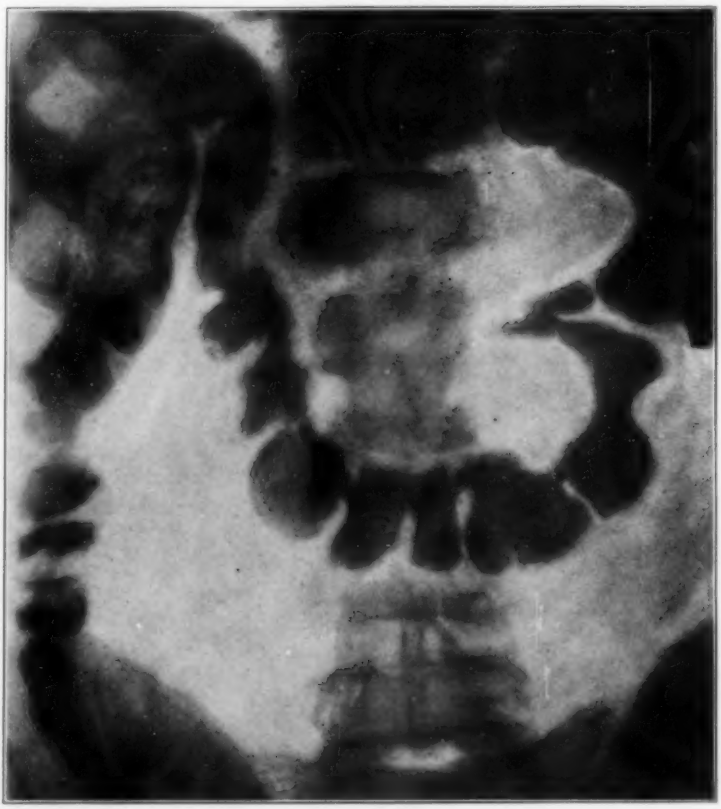

Fig. 2 (Roentgen-Ray Plate 26196).—Bismuth in the large intestine. This shows the prolapse and kinking of the transverse colon in the upper right quadrant, with absence of shadow of the ascending colon on the right side.

the intragastric pressure varied according to the intrathoracic formula in their case of hernia.

When, in Table 2, it is stated that a case exhibited typical physical the following are implied: A normally formed thorax, with somewhat and radiographic signs of eventration of the *left* side of the diaphragm,

diminished expansion on the left side; normal physical signs on the right side, except for the usual evidence of displacement of the heart to that side; normal pulmonary resonance and vesicular breath-sounds over the upper half of the left side, but below the level of the fourth rib, in front and behind, a tympanitic note, absence of voice and breath-sounds, absence of vocal fremitus, and absence of Litten's sign in the left axilla; in place of respiratory murmur, splashing and metallic sounds elicitable over the lower left chest, with great variation in these according to the degree of fulness of the stomach. These physical signs may be given wholly or in part by (1) hydropneumothorax or pyopneumothorax, (2) subphrenic gas abscess, (3) large cavity in the lower lobe of the left lung, (4) paralysis of the diaphragm, and (5) hernia of the diaphragm. The differentiation by simple physical examination between these conditions is almost impossible. Many of the cases of eventration have been explored with the aspirating needle under the supposition of pyopneumothorax (Sailer and Rhein). While the signs here may be similar, the history and general condition of the patient with eventration are different from those features of the other diseases. In eventration, there is not likely to be the sudden onset, great dyspnea and fever that characterizes pyopneumothorax. In subphrenic gas abscess, such as occurs with a walled-off perforation of an ulcer of the stomach, Litten's sign may be still visible in the axilla, while the general condition indicates an acute septic state rarely present in eventration. But in these states, as in hernia, the greatest assistance in diagnosis is derived from investigation by the Roentgen ray.

These differential radiologic findings have been summed up by Becker in a beautifully illustrated paper, as follows:

> In both hernia and eventration a delicate bow-shaped line may be seen arching upward across the left side of the chest. In *hernia*, the contour of this line is irregular, partly distinct, partly blurred; it usually shows a paradoxical respiratory movement; when the stomach and intestines are filled with bismuth, extreme derangement of the line is caused by the new shadows, and when the stomach is inflated with CO_2 the line shows practically unlimited extension upward, along with obvious displacement of the stomach, and the lung shadows can be seen through the bright area of the inflated stomach. In *eventration*, the contour of the line is that of a smooth, sharply defined bow-shaped shadow, with a bright area below and lung-shadows above; the movement is usually normal but diminished; when the stomach and intestines are filled with bismuth, the new shadows take a restricted dome-like position, and when the stomach is inflated there is only a smooth distention, limited by the bow-shaped line, while the lung shadows remain entirely above this line and are not visible through the "stomach-bubble."

While all of these facts together may make the diagnosis almost positive, none is pathognomonic. The diagnosis rests on the phenomena exhibited by the patient as a whole.

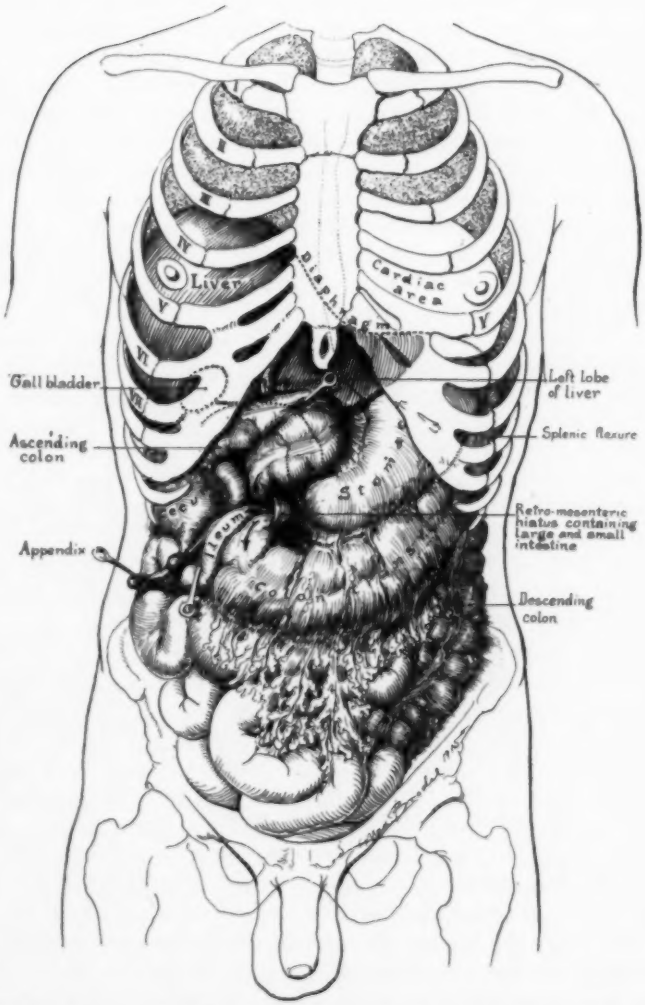

Fig. 3.—Diagram by Max Brödel from sketch at operation. This shows the high-position and eventration of the right side of the diaphragm; the mesenteric anomaly and its hernia; and the malposition of the cecum and ascending colon.

The tendency is to exclude paralysis of the diaphragm, with consequent loss of tone and high position, from the category of eventration. These cases are usually fatal or improve before the typical clinical and anatomical picture of eventration is produced. Important in this connection is the study of the electrical condition of the phrenic nerves by Jamin's method of faradization of the phrenics in the neck. During the stimulation of the nerve, movements of the diaphragm may be observed by fluoroscopy. By this means, the presence or absence of degeneration of the nerve can be determined, and also an estimate of the muscular condition of the diaphragm can be made. These, however, are the limits of the conclusions to be drawn from such observations, and the method cannot be used to differentiate definitely between hernia and eventration.

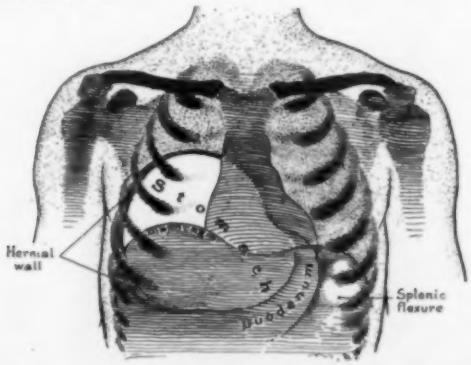

Fig. 4.—Schematic copy of roentgenogram published by Dietlen and Knierim to illustrate Roentgen-ray findings in hernia of the right side of the diaphragm. Here the clear stomach region lies between shadows of the lung above and the liver below. In eventration of the right diaphragm the liver shadow lies between the lung shadow and a bright area due to distended colon in the lower left chest.

PATHOLOGICAL ANATOMY

The pathological anatomy of the diaphragm has been studied at necropsy or operation in twenty-two cases of eventration. In all instances, whether the affection has occurred on the right or the left side, the diaphragm has been found to be a thin, translucent membrane. The peritoneal and pleural reflections have covered the sides of the diseased organs smoothly and uniformly. Between these membranes there has always been found a layer of fibrous tissue, which in some cases contained a few strands of muscle tissue and in others consisted of only a "thin aponeurotic sheet." In the case reported by Doering there was a fringe of normal muscle around the parietal attachments of the diaphragm, disappearing as the dome was reached. In Benda's

case the affected half of the diaphragm was distinguished by its opaque whitish color from the red, muscular aspect of the healthy side. Benda found that the strands of muscle fibers were replaced by parallel bands of fat cells, and he termed the process "lipomatous pseudohypertrophy," correlating it with one of the forms of myogenic muscular atrophy. The pathological anatomy of structures closely related to the diaphragm is variable. The phrenic nerves have been examined in a number of cases of eventration. As in Benda's study, they have been found to be reduced in size in comparison with the nerve of the healthy side, but have been composed of normal fibers, with no great evidence of degeneration in the nerve trunk or its nuclei. The pleura, peritoneum and blood supply of the diaphragm have not shown any abnormalities, and the attachments of the diaphragm have been normal. The lungs and abdominal viscera, however, have exhibited various developmental anomalies, which will be considered when the etiology of the condition is discussed. A significant fact, revealed at autopsy, is that while the diaphragm may be ballooned upward by the abdominal organs, the lungs are not compressed. The heart and mediastinum may be displaced to the right, or left. In fact, displacement of the heart to the right, simulating dextrocardia, is the invariable accompaniment of eventration of the left side of the diaphragm.

ETIOLOGY

When Cruveilhier established the category of eventration of the diaphragm, he regarded the condition as an acquired lesion. Thoma, on the other hand, published the hypothesis that the condition is a congenital anomaly of development. Since then, evidence in favor of each opinion has been adduced, but the majority of the authors, as shown in Table 2, have supported the hypothesis of the congenital origin.

In favor of the acquired nature of the disease, cases have been cited in which the condition has been apparently secondary to (1) pressure differences on the two sides of the diaphragm, (2) disease of the muscle, and (3) disease of the nerves. All of these may follow trauma, but each class embraces several specific diseases. It has been demonstrated that elevation of the dome of the diaphragm follows increased abdominal pressure. The chief states producing this are pregnancy, ascites, and chronic gastro-intestinal distention, such as meteorism and Hirschsprung's disease. If this factor of increased pressure in the abdomen bore an important etiological relationship to eventration, the frequency of pregnancy in general—and sometimes in the individual—should find a counterpart in a large number of women suffering from eventration of the diaphragm. Table 2, how-

ever, shows a surprising minimum of females among those presenting this affection. Among the other dynamic intra-abdominal causes of eventration, great importance has been attached by Hoffmann to chronic gaseous distention of the intestines in his hypothesis that *"chronische Magenblase"* produces at least "rudimentary eventration." His conception is that pressure on the diaphragm produces circulatory disturbances which lead to "loss of elasticity," degeneration of the muscle, and stretching of the organ. While it is conceded that "high position" may be produced in this way, it is doubtful whether the condition found in eventration actually results from this process. A strongly unfavorable criticism of this hypothesis is that bulging of the abdominal wall and ptosis of the viscera are the most common sequels of increased abdominal pressure, while in eventration, the tonus of the abdominal wall is at least normal. From the pulmonary side, retracting pleural adhesions may drag the dome of the diaphragm upward. Thoma describes a case in which this occurred, but he uses it as a contrast to true eventration, from which category he eliminates it, along with similar cases of mechanical *"hochstand"* of the diaphragm.

The question of the etiological significance of disease of the muscle and nerves in eventration of the diaphragm is more difficult to settle. It is well known that in progressive muscular atrophy, the diaphragm becomes so degenerated and weakened that its loss of function may be the cause of death. But here, while the state of the diaphragm may resemble that seen in some cases of eventration, its evident part in a general disease differentiates it from the localized diaphragmatic lesion of eventration. In Benda's case, for example, although there was a "lipomatous pseudohypertrophy" of the atrophied muscle, there was no accompanying nerve lesion. Peripheral neuritis of the phrenic nerve, after diphtheria and other infectious diseases, and in toxic states like alcoholism and beriberi, has produced relaxation of the diaphragm and consequent high position. In practically all of these cases, however, the normal tone and position of the diaphragm has been regained when the nerve lesion improved (Behrenroth), or if death has occurred, the diaphragm has not shown the flaccid fibrosis characteristic of eventration.

The criteria of the congenital origin of eventration, as noted by Thoma in 1882, have gained validity from the studies of subsequent investigators. These points are chiefly: (1) the coincidence of the frequency of eventration of the left side of the diaphragm with the complexity of the development of that side of the organ, (2) the occurrence of eventration in the fetus and the new-born, (3) its association with other congenital anomalies of the body, and (4) the usual absence of symptoms over long periods of time. Detailed

recapitulation of the embryology of the diaphragm would be more confusing than useful here, where all that need be emphasized is that the left side of the organ develops from a number of sources and is not entirely formed until some time after the completion of the relatively simple development of the right side. On the right, the liver, growing in the septum transversum, which early separates the pleural and peritoneal cavities on that side, seems to guard the right side of the diaphragm from developmental disturbances. The left side, however, grows in close association with the left lobe of the liver, the stomach and spleen, the pericardial formation, one of the pulmonary ridges, and the pleuroperitoneal membrane. In addition, the posterior portion of the left side of the diaphragm is the last to close. This undoubtedly accounts for the great predominance of congenital hernia of the left side of the diaphragm. By analogy, the great relative frequency of eventration of the left side suggests not only that this condition is associated with the developmental intricacy of that side, but also that it is a congenital abnormality. The finding of eventration in either fetuses or young children in four of the forty-five cases of the disease is good evidence in favor of its congenital origin (see Cases 1, 3, 25, 35 in Table 2). In nine cases (Cases 8, 10, 12, 13, 15, 16, 40, 45, Table 2), other abnormalities of the lungs or abdominal viscera were present. These were abnormally lobulated lungs, "stunted lungs," congenital hernias, and true dextrocardia. In the case described in this paper, the deformity of the mesentery and the position of the cecum under the right costal margin were clearly congenital abnormalities. This coincidence of rare anomalies suggests a common etiological relationship; namely, that eventration also is a congenital dystrophy. In connection with recent studies of body growth, Byloff has published papers on the thesis of high position of the diaphragm as a stigma of degeneration. He found moderate high position in a number of individuals who showed defective development of various organs and parts of the body. None of Byloff's examples is a case of eventration, but his work is capable of interpretation in favor of the congenital origin of this affection.

It has been noted, in the discussion of the pathological anatomy of eventration, that the lung lying above the distended diaphragm has not been compressed, although its thoracic chamber has been always greatly reduced. This uniform absence of compression of the contiguous lung is to be emphasized here as one of the strongest arguments in favor of the congenital origin of eventration of the diaphragm.

PROGNOSIS

It is probable that if the degree of eventration be extreme in the new-born, this condition is incompatible with life. After the first year, however, the disease is rarely a cause of death. While the

greatest number of cases have been found in individuals between the ages of 20 and 50 years, instances have been observed in patients in the eighth decade of life. Cardiorespiratory diseases have not been unusually frequent in those suffering with eventration of the diaphragm, but gastro-intestinal symptoms have been the most distressing feature. The danger of incarceration of the displaced abdominal viscera is the only element which renders the prognosis grave.

SUMMARY

This paper reports a case of eventration of the right side of the diaphragm. This diagnosis, made from physical examination, is the first of its kind determined during the life of the patient. The clinical impression was confirmed at an operation.

Tabulation is made of forty-five cases of eventration of the diaphragm. Of these, three were right-sided, while forty-two were left-sided lesions.

In the differential diagnosis between eventration and allied states, the difficulties are pointed out, particularly with regard to the differentiation from hernia of the diaphragm. No single method is capable of establishing this differentiation, but all together render the diagnosis reasonably certain.

The various etiological hypotheses are summarized, showing that the weight of evidence is in favor of the opinion that the disease has a congenital origin.

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A STUDY OF THE LIPIN CONTENT OF A CASE OF GAUCHER'S DISEASE IN AN INFANT*

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Lipoid bodies play an important part in many pathologic processes, especially in those involving the spleen and lymph nodes and other blood-forming organs which, until recently, were not suspected of bearing any relation to the distribution of the lipins of the body. Cells which contain considerable lipoid substances are usually characterized, in routine histologic sections, by the relatively large amount of pale, more or less uniformly vacuolated or honeycombed cytoplasm, and small rather deeply stained and often eccentrically placed nuclei. The vacuoles in the cytoplasm represent spaces left by lipoid material that has been dissolved during embedding. In fresh tissue this material does not react typically to the various microchemical reactions for neutral fat. Clusters of such lipoid cells have been frequently noted in chronic inflammations such as chronic aortitis and chronic salpingitis, especially where there is considerable lymphoid infiltration (Pick,[1] Krompecher[2]); but it is only in the recent work of Poscharisky[3] and Kusunoki[4] that fats and lipoids are described in considerable amounts in the spleen in association with various pathologic processes and, indeed, even in some normal conditions. This accumulation of lipoids in the spleen and other blood-forming organs promises to contribute considerably to the elucidation of Gaucher's disease and allied xanthelasmic processes in which the parenchyma of the hematopoietic organs is more or less replaced by distinctive large pale cells whose cytoplasm tends to be vacuolated.

Gaucher[5] described the first case of the disease named after him in 1882, and noted particularly the large pale cells which he considered

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1. Pick, Ludwig: Uber besondere Structuren in alten Eitertuben, Berl. klin. Wchnschr., 1908, No. 37.

2. Krompecher: Vergleich biologish-morphologische Studien betreffend die Fibroblasten und Makrophagen (Eiterphagocyten, Pseudoxanthom-, Typ Gaucher-, Malakoplakie-, Rhinoskleronzellen) des menschlichen Granulationsgewebes, Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's) 1913, lvi, 346.

3. Poscharisky, J.: Zur Frage des Fettgehaltes der Milz, Beiträge, z. path. Anat. u. z. allg. Path., 1912, liv, 369.

4. Kusunoki, Masanobu: Lipoidsubstanzen in der Milz und im Lichenblut, Beitr. z. path. Anat. u. z. Allg. Path., 1914, lix.

5. Gaucher: De l'epithélioma primitif de la rate, Paris, 1882.

epithelial in origin. Since then about eighteen additional cases, confirmed by histologic examination, have been reported, resulting in considerable discussion and dispute over the origin of the large pale cells.

Marchand[6] was the first to note the presence of a peculiar homogeneous substance within the cells which is dissolved in alcohol, thus explaining the vacuolated appearance of the cells in the paraffin sections. Further, he concludes that there is "involved, therefore, not a real protoplasmic hyperplasia or even a tumor growth, but in the main a deposit of a foreign substance which, under certain treatment, leaves behind a vacuolated meshwork."[7]

Risel[8] studied the same case and confirmed Marchand's findings.

The next advance was made by Schultze[9] in his report of a case of diabetic lipoidemia associated with marked "Lipoidzellenhyperplasie" of the spleen with almost complete replacement of the pulp by these large pale cells. He pointed out that the vacuolated appearance of the cytoplasm was due to a deposition of a homogeneous substance of a lipoid character, probably closely related to cholesterol. After examination of material from Risel's a..d Schlagenhaufer's cases he came to the conclusion that "in my case the same cells are concerned as in the case of Risel and Schlagenhaufer. They agree in size and in staining reaction to embedded material."[10] While the cells were alike he did not attempt to identify the two conditions as from the same process, but suggested that, in Gaucher's disease, the vacuolated cytoplasm of the cells was also due to the deposition of lipoid substances. In his case he concluded that the cells were transformed reticular cells.

In his report of two cases of large-celled hyperplasia of the spleen pulp (*grosszellige Hyperplasie der Milzpulpa*) in diabetic lipemia, Lutz[11] showed that his cases are identical with Schultze's case, the cells

6. Marchand: Ueber sogenannten idiopathische Splenomegalie (Typus Gaucher), München. med. Wchnschr., 1907, liv, 1102.

7. Also nicht um eine eigentliche protoplasmatische Hyperplasie, noch weniger um eine Geschwulstbildung, sondern in der Hauptsache um Einlagerung einer fremdartigen Substanz zu handeln, die bei gewissen Behandlungen ein vakuoläres Maschenwerk zurücklässt.

8. Risel, W. Ueber die grosszellige Splenomegalie (Typus Gaucher), Beitr. z. path. u. z. allg. Path., 1907, xlvi, 241.

9. Schultze, W. H.: Ueber grosszellige Hyperplasie der Milz bei Lipoidaemie (Lipoidzellenhyperplasie), Verhandl. d. deutsch. pathol. Gesellsch., xv, Tagung 1912, xv.

10. Dass es sich bei meinem Falle um die gleichen Zellen wie dort handelt. Sie stimmen in der Grösse und dem Färberischen Verhalten an eingebettetem Material überein.

11. Lutz, Wilhelm: Ueber grosszellige Hyperplasie der Milzpulpa bei diabetischer Lipämie, Beitr. z. path. Anat. u. z. allg. Path., 1914, lviii, 273.

being filled with lipoid substances. He also studied the material from the cases of Gaucher's disease reported by Risel, Schlagenhaufer and by DeJong and von Heukelom, and confirmed Schultze in his conclusion that, with a few minor exceptions, the large pale cells were identical in the two conditions and contained deposits of lipoid material; but he pointed out that the identity of the cells does not imply that the processes are identical. He also noted three types of lipoid cells, each reacting slightly differently to the microchemical reactions for lipoids and fats, and only one containing an anisotropic substance. Most of the cells contained only isotropic material. This would indicate that the lipoid substances are not identical. He believed that the lipoids resulted from the transformation of fats deposited in the reticular and endothelial cells.

Mandelbaum and Brill[12] failed to obtain any positive reaction for neutral fat, cholesterin or cholesterin ester in the fresh material from a case (Bernstein's) of Gaucher's disease, but their report on this case has not been completed.

In the past year Sapegno[13] reported two cases of Gaucher's disease in which he concluded that there was a deposit of lipoids in the characteristic large pale cells, which the author considered as transformed embryonic lymphoid cells. He noted the similarity of Gaucher's disease and the cases of lipoidemia with large accumulations of lipoid cells in the spleen, but pointed out that this did not insure the identity of the two conditions. He referred to Kramer's case of obstructive jaundice in which masses of lipoid cells were described in Glisson's capsule of the liver and in the splenic trabeculae; and he criticized Aschoff's classification of these allied conditions under xanthelasmic diseases in three divisions, as being premature and artificial. He suggested the following classification of xanthelasmic diseases as a temporary one until more is found out about these processes:

(a) Lipoid infiltration of specific elements (Gaucher's disease).

(b) Lipoid infiltration of the cells of the reticulum and endothelium (Schultze-Lutz type).

(c) Lipoid infiltration of purely connective tissue elements (Kramer).

Anitschkow[14] has produced experimentally, in rabbits, a condition similar to Gaucher's disease by prolonged feeding of cholesterol, in

12. Brill, N. E., and Mandelbaum, F. S.: Large Cell Splenomegaly (Gaucher's Disease), Am. Jour. Med. Sc., 1913, cxlvi, 863.

13. Sapegno: La Spenomegalia tito Gaucher e le Splenomegalie lipoidemicat, La Pedriatica, xxii, 606.

14. Anitschkow: Ueber experimentelle erzeugte Ablagerungen von anisotropen Lipoidsubstanzen in der Milz und im Knochenmark, Beitr. z. path. Anat. u. z. allg. Path., 1914, lvii, 201.

that the parenchyma of the spleen, lymph nodes and bone marrow is replaced by large pale vacuolated cells containing large deposits of cholesterin ester. The picture in the spleen resembles very closely that described by Schultze and Lutz. The latter noted this close similarity. The deposit occurs in the endothelial and reticular cells. While Anitschkow noted the resemblance to Gaucher's disease, he believed that, at present, a complete analogy is not warranted, though there is a systemic involvement in the rabbits, in that all organs involved are provided with those cell elements (reticular cells, endothelial cells, Kupfer cells, etc.) which have essentially the same functional significance.

The case[15] on which our study is based is that of an infant 11 months of age, with a clinical picture which, in general, simulated Gaucher's disease. In many respects it bore a close resemblance to Niemann's case.[16] The spleen, liver and lymph nodes presented the usual changes, but the unusual feature of the case was the almost complete substitution of the medulla of both suprarenals by clusters of the large pale vacuolated cells. The latter were also present in Peyer's patches, in the intestines and in the thymus, besides involving the adventitia of some of the smaller vessels. The process was thus much more diffused than in any case hitherto described, and also the first one described in an infant, when the condition may be more diffused than when it occurs in adults.

MICROCHEMICAL STUDY

Examination of frozen sections of the fresh tissue and of tissue fixed in liquor formaldehydi showed the presence of many small homogeneous droplets in the cytoplasm of most of the cells, giving it a coarsely granular appearance under low magnification. These droplets disappeared on treatment with alcohol or ether, leaving the vacuoles which are so prominent in the paraffin and celloidin sections. A large number of the cells did not show these droplets, or else they were so small that they appeared as small granules. Acids and alkalies had no effect on the droplets. With the polarized light, many of the cells in the spleen had a luminous appearance with here and there a small highly refractile granule. In addition, there were small clusters of cells which, in the spleen, tended to group in and about the malpighian bodies, and which contained a large number of anisotropic droplets, some of the larger ones showing dark crosses in the center.

15. A detailed report of this case, associated with that of the sister of our patient, will be published shortly in the Bulletin of Johns Hopkins Hospital.

16. Niemann: Ein unbekanntes Bild der Kinder, Jahrb. d. Kinderh., 1914, lxxix, 1.

Some of the smaller ones were needle-shaped. On warming, most of these doubly refractile substances either became very obscure or entirely disappeared, and reappeared on cooling. While most of these bodies were within the large pale cells, some were found between them. While cells with isotropic droplets in the cytoplasm greatly predominated in the spleen and liver, cells containing anisotropic droplets were more abundant and more diffusely distributed in the lymph nodes and, especially, in the medulla of the suprarenal gland. This reaction toward the polarized light would indicate the presence of cholesterol or one of its compounds,[17] but the Golodetz reaction for

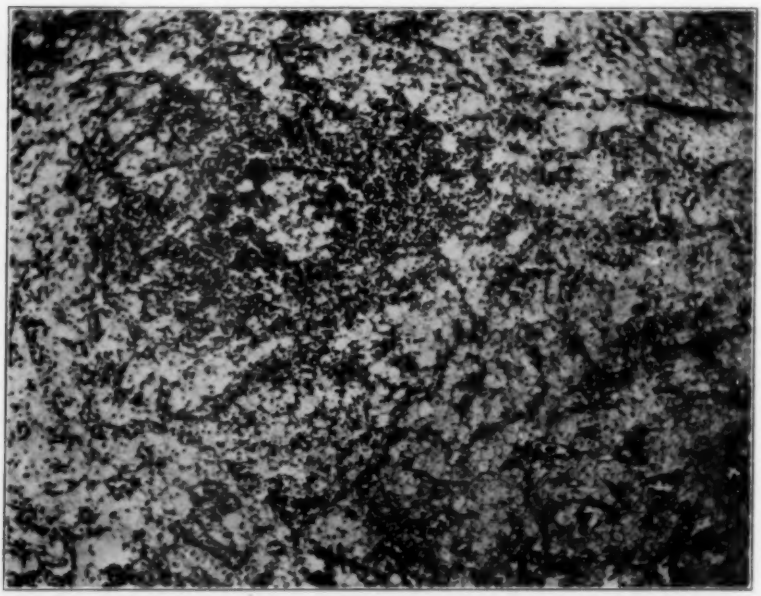

Fig. 1.—Photomicrograph of the spleen, × 85, stained with iron hematoxylin, showing the more or less extensive replacement of the spleen pulp by the distinctive large pale cells. Note that some of the cells are also present in the center of a malpighian body.

cholesterol was negative. Scharlach R and sudan III stained the large pale cells a faint orange or not at all, whereas the neutral fat in control sections took a deep orange-red. With osmic acid the cells took a very faint gray. With nilblau sulphate the droplets in the cytoplasm

17. Aschoff: Zur Morphologie der lipoiden Substanzen, Beitr. z. path. Anat. u. z. allg. Path., 1910, xlvii, 1.

of the distinctive large cells in the spleen and liver stained from a violet to a pale blue. In one section of the spleen a faint pink was obtained. In the lymph nodes the color was either violet or pink. In the medulla of the suprarenal glands a definite pink was invariably obtained. The droplets in the cortex stained violet or blue. Weigert's hematoxylin (Schultze[9]) showed deep blue droplets in the large pale cells. Lorain-Smith-Dietrich's stain for lipoids was also positive in most of the large cells. Weigert's myelin sheath stain was only partly positive in that the droplets were not entirely decolorized. The material did not stain with neutral red, or with trypanblau or trypanrot. Fischler's stain for fatty acids and soaps was negative. With Ciaccio's stain the results were at first negative, but after the time in chrome salts had been increased and after preliminary treatment with Carlsbad salt, in accordance with Kasarinoff's suggestions,[18] faint but very definite pale orange droplets appeared in the large pale cells in the spleen. This would indicate the presence of lecithin (Ciaccio[19]), but it is more probable that other lipoids are equally responsible for the reaction (Polosow[20]).

The foregoing microchemical reactions are not entirely satisfactory, being incomplete or poorly defined in some cases. Very little neutral fat is present. The anisotropic reaction and pink color in the suprarenal medulla and some parts of the spleen indicate the presence of cholesterol or one of its compounds, while the isotropic material and the violet or pale blue color with nilblau implies the presence of other lipoids such as lecithin and phosphatids. The variation and incompleteness of the other reactions is accounted for, in part, by the probable presence of several different lipoid substances and, in part, to alterations produced by the formaldehyd fixation, since all of the material at hand was fixed in 10 per cent. dilution of liquor formaldehydi. Both Bell[21] and Bullard[22] have shown that liquor formaldehydi materially interferes with the reactions for lipoids. On the other hand, Kassarinoff[18] showed that preliminary treatment of formaldehyd-fixed tissue in Carlsbad salt renders the lipoids more susceptible to the lipoid stains, and we noted the same improvement in following this suggestion. Risel and Marchand did not note any

18. Kasarinoff: Verglichungen zur Histologie der Lipoide, 1910, xlix, 490.

19. Ciaccio, Carmelo: Ueber das Vorkommen von Lecithin in den zellularen Entzündungsprodukten und über besondere lipoidbildende Zellen (Lecithinzellen), Zentralbl. f. allg. Path. u. path. Anat., 1909, p. 385.

20. Polosow, A. A.: Zur Morphologie der Lipoidsubstanzen, Zentralbl. f. allg. Path. u. path. Anat., 1910, p. 1014.

21. Bell, E. T.: Staining of Fats in Epithelial Cells and Muscle Fibers, Anat. Rec., 1910, iv.

22. Bullard, H. Hays: The Microscopical Demonstration of Fats in Tissue Sections, Jour. Med. Research, 1913, xxvii, 55.

change in the material in the cells of their cases as a result of fixation. In our case the presence of many isotropic droplets in the cells and comparatively few anisotropic droplets may be the result of the transformation of the latter into the former by the action of liquor formaldehydi, as was shown by Dunin-Karwicka[22] and Aschoff.[17]

These liquid crystals and other anisotropic particles are undoubtedly cholesterol or some derivative (Aschoff,[17] Dunin-Karwicka,[23]

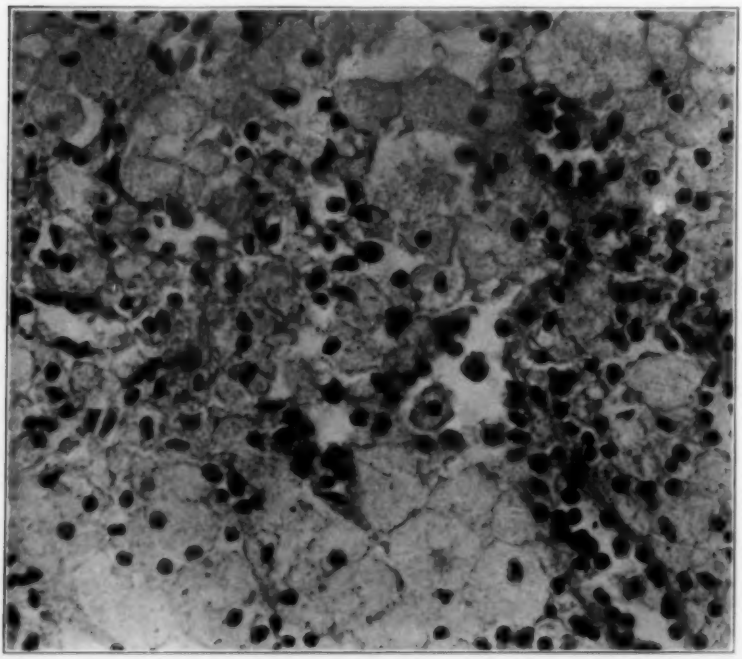

Fig. 2.—Photomicrograph from the same areas as in Figure 1, but with a magnification of 450, showing the character of the large cells and the vacuoles that are present in the cytoplasm.

Chalatow[24]). Most of the cells did not contain anisotropic particles, but Aschoff[17] pointed out that the absence of doubly refractile substances does not exclude cholesterol. The predominance of violet or

23. Dunin-Karwicka: Ueber das physikalische Verhalten und das physiologische Vorkommen der doppeltbrechenden Lipoide, Beitr. z. path. Anat. u. z. allg. Path., 1911, L, 437.

24. Chalatow: Ueber Myelinosis und Xanthomatosis, Virchows Archiv. f. path. Anat., 1914, ccxvii; ueber flüssige Kristalle in tierischen Organismus, Frankfurt. Ztschr. f. Pathol., 1913, xiii, 189.

pale blue cells over the pink ones with nilblau is one point that would suggest a corresponding predominance of lecithin and phosphatids over cholesterol. Even at best, the staining reactions are notoriously unreliable for careful differentiation of the various lipoids and fats and, in formaldehyd-fixed tissue, this defect would be enhanced. However, the conclusion that is justified from the histochemical study of the tissue is that the material, accumulated in the large pale cells, is not a protein or a neutral fat, but is composed of fatlike bodies—lipoids, including cholesterol and lecithin. The relative amount of cholesterol as compared to lecithin and the phosphatids is uncertain.

Owing to the unreliability of the fat and lipoid stains in the differentiation of the lipins, it occurred to us that more definite information about the material accumulated in the large pale cells could be obtained by extracting the liver and spleen for lipoids, quantitatively. The great difficulty with this procedure is again the fact that all of the material at our disposal had been fixed in liquor formaldehydi, and it is well known that lipoids and even neutral fats are more or less altered by this solution. However, the extent of this alteration, quantitatively, has not, to our knowledge, been determined, and it was suggested that if the same technic was carried throughout with proper control organs and Gaucher organs, a general conception of the main changes in the distribution and relation of the lipins in Gaucher's disease may still be obtained.

The quantitative method for the determination of lipoids used was one elaborated in accordance with the suggestions of Frankel[25] and Erlandson,[26] and depended on the initial extraction with petroleum benzin followed by extraction with alcohol. The technic and method were identically the same for all determinations and were briefly as follows:

The organs used were the spleen and liver. Extraction was made only from the dried tissue. *A.* The tissue was first extracted in a Soxhlet extractor and reflux condenser for thirty-six hours with petroleum benzin of a low boiling point (less than 37 C.). This removed practically all of the fat and lecithin and a large part of the cholesterol and phosphatids. *B.* The tissue was then extracted thirty-six hours with hot 95 per cent. alcohol. This extract contains a part of the cholesterol and phosphatids. By means of saponification with alcoholic sodium hydroxid, the phosphatids were mostly saponified, and the residue of unsaponified material consisted chiefly if not entirely of cholesterol. *C.* The petroleum benzin extract was treated with a large amount of cold acetone. The precipitate of acetone-insoluble material is probably composed mainly, if not entirely, of lecithin. *D.* The filtrate from *C*, which contained all of the

25. Frankel, Sigmund: Darstellung von Lipoiden aus Gehirn und anderen Geweben. Abderhalden: Handbuch der biochemischen Arbeitsmethoden, 1911, v.

26. Erlandson, cited by Bang, Ivar: Chemie und Biochemie der Lipoidsubstanzen, Wiesbaden, 1911.

fixed fat and a part of the cholesterol and phosphatids, was evaporated to a small volume and 95 per cent. alcohol added in excess. The fixed fat is not dissolved, while the cholesterol and phosphatids go readily into solution. *E.* The amount of fixed or neutral fat in *D* is obtained by saponification. *F.* The filtrate from *D*, that is, the phosphatids and cholesterol, was saponified and the cholesterol roughly separated from the phosphatids as it cannot be saponified. These quantities were then added to similar quantities in *B*. *G.* The lecithin or acetone-insoluble material was redissolved in ether, the solvent evaporated, the residue dried to a constant weight and then saponified to check the result found gravimetrically. In each of the foregoing steps the solvent was evaporated spontaneously, and the residue dried in a desiccator to a constant weight

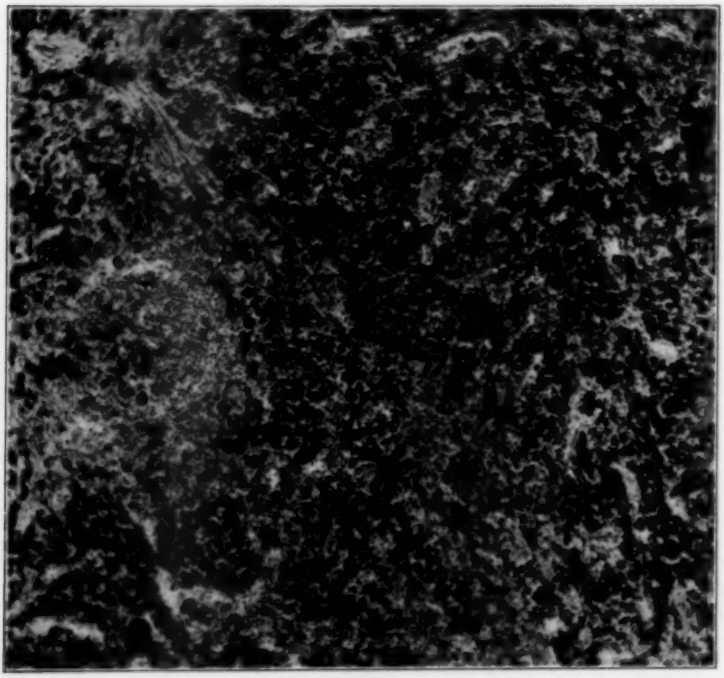

Fig. 3.—Photomicrograph of a preparation, × 85, stained for lipoid material after Schultze's chrome hematoxylin method. The large pale cells are represented by irregular rounded black masses owing to their being filled with lipoid material, which takes a deep steel-blue color.

and the weight recorded. The saponification was not made in all of the determinations, it being considered unnecessary, inasmuch as it only afforded a check on the gravimetric values.

This method is not perfect, but the errors that occur are common to both the Gaucher tissue determinations and those on the control organs. For controls, fresh tissue from the spleens and livers,

TABLE 1.—Lipoids in Control and Gaucher Tissue

Organs	Determination	Total Weight of Dried Material, Gm.	Terms	Lecithin (Acetone-Insoluble Portion)		Cholesterol, Phosphatids, etc.						Total			Fats (fixed)	Total Lipins
						Alcohol-Soluble Portion of Acetone Filtrate			Direct Alcoholic Tissue Extract							
				By Precipitation	By Saponification	Total	Saponifiable (Lecithin, Phosphatids, etc.)	Non-saponifiable (mostly Cholesterol)	Total	Saponifiable (Lecithin, Phosphatids, etc.)	Non-saponifiable (mostly Cholesterol)	Saponifiable (Lecithin, Phosphatids, etc.)	Non-saponifiable (mostly Cholesterol)	Grand Total		
Normal spleen	Case 1	3.617	Grams...	.0715	.....	.2544	.2515	.0029	.2934	.2934	0	.5449	.0029	.5478	.1644	.7837
			Per cent.	1.97	.....	7.	6.94	.06	8.11	8.11	0	15.05	.08	15.13	4.5	21.55
	Case 2	2.091	Grams...	.061	.....	.193	.....	.....	.316	1.03	.123	?	?	.509	.006	.576
			Per cent.	2.9	.....	9.23	.....	.....	15.12	9.23	5.9	?	?	24.3	.3	27.5
	Case 3	4.26	Grams...	.147	.....	.1845	.....	.....	.347	.....	.....	?	?	.5315	.022	.7005
			Per cent.	3.45	.....	4.33	.....	.....	8.14	.....	.....	?	?	12.47	.52	16.44
	Average	.....	Per cent.	2.77	.....	6.85	.....	.....	10.45	.....	.....	.....	.....	17.30	1.77	21.84
Gaucher spleen	Det. 1	2.418	Grams...	.355	.255	.3188	.2032	.1256	Lost	Lost	Lost	.2032	.1256	.3288	.0066	.7498+
			Per cent.	14.3	10.2	13.2	8.16	5.06	Lost	Lost	Lost	8.16	5.06+	13.2+	.26	27.76+
	Det. 2	2.717	Grams...	.2246	.....	.5229	.214	.3089	.632	.384	.243	.558	.556	1.154	.....	1.378
			Per cent.	8.43	.....	19.3	8.	11.3	23.3	14.16	8.14	22.16	19.44	41.6	.....	50.03
	Det. 3	1.792	Grams...	.202	.157	.373	.328	.055	.388	.237	.151	.565	.206	.771	.0229	.996
			Per cent.	11.28	8.76	20.8	18.3	2.5	21.8	13.2	8.6	31.5	11.1	42.6	1.2	54.08
	Average	.....	Per cent.	11.33	9.48	17.76	11.35	6.28	22.5	13.68	8.37	26.83	15.27	42.1	.73	52.05
Normal liver	Case 1	5.00	Grams...	.0824	.0732	.274	.295	0	.694	.585	.109	.880	.109	.989	.490	1.561
			Per cent.	1.64	1.46	5.49	5.9	0	13.88	11.7	2.19	17.6	2.19	19.8	9.8	31.16
	Case 2	5.004	Grams...	.177	.....	.225	.....	.....	.443	.....	.....	.....	.....	.668	.0520	.945
			Per cent.	3.5	.....	4.45	.....	.....	8.75	.....	.....	.....	.....	13.2	1.03	17.73
	Case 3	2.874	Grams...	.48	.....	.236	.....	.....	.293	.....	.....	.....	.....	.529	.084	.661
			Per cent.	2.01	.....	8.2	.....	.....	10.2	.....	.....	.....	.....	18.4	2.74	23.15
	Average	.....	Per cent.	2.38	.....	6.05	.....	.....	10.94	.....	.....	.....	.....	17.13	4.52	24.03
Gaucher liver	Det. 1	2.3274	Grams...	.2357	.....	.435	.2139	.2213	.1194	.....	.....	.....	.....	.5544	.002	.782
			Per cent.	10.11	.....	18.6	9.2	9.5	5.12	.....	.....	.....	.....	23.22	.09	33.9
	Det. 2	4.906	Grams...	.550	.248	.8506	.38	.47	.488	.2	.288	.58	.758	1.338	.09	1.978
			Per cent.	11.21	5.05	17.33	7.75	9.57	9.94	4.08	5.87	11.83	15.24	27.07	2.	40.48
	Average	.....	Per cent.	10.66	5.05	17.96	8.97	9.43	9.94	4.08	5.87	11.83	15.24	27.07	1.04	37.19

Note.—Through the kindness of Drs. J. H. Mason Knox and of H. C. Schmeisser of Baltimore, material from an infant sister of our patient with a very similar history and pathologic picture was placed at our disposal. The microchemical reactions on the fresh material were the same as in our formaldehyd-fixed material except Ciaccio's stain, which was more strikingly positive in the former. But one quantitative determination on the fresh liver material was made with the following data: total dried material, 1.9715 gm.; acetone precipitable bodies (lecithin), 0.237 gm. or 12.03 per cent.; cholesterol, 0.744 gm. or 32.7 per cent.; phosphatids, 0.247 gm. or 12.6 per cent.; total cholesterol and phosphatids, 0.990 gm. or 50.9 per cent. The fat moiety was partly lost, and for that reason was not calculated. These data in general confirm the conclusions based on those obtained in our case.

obtained at random from the necropsy room, were used. Inasmuch as no complete quantitative analysis of the different lipoids in the normal human spleen or liver has been published to our knowledge, our determinations afford a general conception of their distribution and relation.

The results of the determinations are condensed in the accompanying tables. Table 1 includes the data of the determinations arranged so that those on the Gaucher material may be readily compared with those on the control tissue. From this table it is seen that there is a very striking uniform increase in the lecithin (or rather the acetone-insoluble residue) in the Gaucher material as compared with that in the control organs. There is from three to four times as much in the former as in the latter. For example, in the Gaucher spleen there is an average of 11.33 per cent. of dried substance or 21 per cent. of total lipins as compared to 2.77 per cent. of dried substance and 12 per cent. of total lipins for an average of three different spleens. For the livers the proportion of lecithin is still higher, being 26 per cent. of the total lipins as compared to less than 10 per cent. for normal livers. There is also a marked increase in the cholesterol in the Gaucher tissue, but our data on the normal material are not sufficiently complete on this point to make them reliable. The phosphatids do not show such a marked increase, while the fixed fats in the Gaucher tissue are markedly diminished. The total lipins are considerably increased (from 60 to 100 per cent.), but not as much proportionately as the lecithin. The high fat content of the control spleen and liver of the first case is readily accounted for by the fact that these organs were taken from a case of pulmonary tuberculosis in which all of the abdominal viscera showed considerable fatty change.

Table 2 shows the result of a series of determinations made to show the effect of liquor formaldehydi on the extraction of lipoids and fats after our method. These determinations were made on the fresh tissue and formaldehyd-fixed material of the same cases which were as near normal and as near the age of our case of Gaucher's disease as we could obtain. The data are arranged together so that a comparison between the unfixed and formaldehyd-fixed tissue may be readily made. It is at once evident from this table that while liquor formaldehydi does cause some alteration, it is so small that it is practically negligible when compared with striking changes as are present in the Gaucher tissue. Throughout this series of determinations, the values for the formaldehyd-fixed tissue are slightly higher than for the unfixed material. This may be explained by either the liberation of some fats firmly bound in protein combination by liquor formaldehydi or, more probably, by the addition of a formol radical to the lipoid substances. It is well known that with lecithin this combination occurs, and it would

TABLE 2.—Lipoids in Fresh Material and Material Fixed in Liquor Formaldehydi

			Spleen						Liver					
			Fresh Tissue		Average	Tissue Fixed in Liquor Formaldehydi		Average	Fresh Tissue		Average	Tissue Fixed in Liquor Formaldehydi		Average
						3 mo.	2 mo.					3 mo.	2 mo.	
Case			1558	1563	.....	1558	1563	.....	1558	1563	.....	1558	1563	
Amount of dried material			1.498	2.523	.....	1.225	1.802	.....	2.874	2.508	.....	1.328	1.889	
Lecithin (Ppt. by acetone)		Grams	.020	.055	.....	.018	.044	.....	.038	.088	.....	.053	.040	
		Per cent.	1.33	2.17	1.76	1.46	2.22	1.80	2.01	3.5	2.85	2.48	2.11	2.28
Cholesterol, phosphatids, etc.	Alcohol-soluble portion of acetone filtrate	Grams	.027	.189	.....	.100	.115	.....	.236	.1051?	.....	.114	.169	
		Per cent.	1.8	7.4	7.4	8.16	6.08	7.12	8.2	2.	8.2	8.6	8.9	8.75
	Direct alcoholic extract of tissue	Grams	.159	.225	.....	.129	1.43	.....	.298	.262	.....	.184	.237	
		Per cent.	10.6	8.9	9.7	10.5	8.09	9.28	10.2	10.4	10.3	13.9	12.5	13.2
	Total	Grams	.186	.414	.....	.229	.258	.....	.529	.313	.....	.298	.406	.....
		Per cent.	12.4	16.3	14.3	18.66	14.17	16.41	18.4	12.4	15.5	22.5	21.4	21.95
Fixed fats (saponifiable)		Grams	.0141	.0772	.....	.0187	.096	.....	.0845	.....	.....	.0876	.095	
		Per cent.	.93	3.05	1.99	1.5	4.77	3.13	2.74	.....	2.74	2.82	4.2	3.51
Total lipins		Grams	.220	.546	.....	.365	.398	.....	.6715	.401?	.....	.368	.541	.....
		Per cent.	14.66	21.42	18.02	21.62	21.26	21.44	23.15	15.9?	24.09	27.80	27.71	27.76

account for the slightly increased amount of lecithin obtained in our determinations. It is only in the case of fixed fats that the change is very considerable.

In Table 3 the results of our determinations are summarized in terms of percentages so that they are more easily interpreted. Inasmuch as the Gaucher tissue was fixed in liquor formaldehydi there is, in this table, also a series of determinations on two formaldehyd-fixed controls for comparison. This table shows that the combined total of cholesterol and phosphatids in the Gaucher spleen is markedly increased over that in the control spleens, while in the liver the increase was not nearly so prominent. Their percentage of the total lipins is not materially changed. The lecithin again shows a very striking increase in both organs in Gaucher's disease, as shown in Table 1, while the fixed fat is decreased.

The results of this quantitative analysis lead us to conclude that there is a very marked alteration in the relation and distribution of the lipins in our case of Gaucher's disease, and that there is a striking increase in the acetone-insoluble material (which is most probably lecithin and lecithin-like bodies), a marked accumulation of cholesterol or one of its compounds; a relative diminution in the phosphatids and a very considerable decrease in the quantity of fixed fats. They also show that, exclusive of the fixed fats, the alteration of the lipins produced by formaldehyd fixation does not materially interfere with their extraction, but owing to the paucity of our determinations not much stress can be placed on this point.

SUMMARY AND COMMENT

The microchemical study of our case indicates not only the accumulation of lipoids in the large pale cells but also that these bodies are not identical. Many of the cells contain droplets that give reactions for cholesterin ester, that is, were doubly refractile to polarized light, stained pale orange with sudan III and pink with nilblau sulphate. Other cells appeared as if covered with a luminous dust which Chalatow[24] showed was due to the accumulation of very small liquid crystals. These are formed wherever fat is present from the union of fatty acid and cholesterol.[24] However, most of the cells did not show anisotropic particles, and gave a violet or pale blue color with nilblau, a faintly positive reaction for Ciaccio's stain, a positive reaction for the Lorain-Smith-Dietrich reaction for lipoids and also for myelin, suggesting the presence of other lipoid bodies. The fact that formaldehyd fixation may, especially if prolonged, interfere with the various microchemical reactions for lipoids and with the action of polarized light makes the interpretation of the results of these methods on for-

TABLE 3.—Comparative Table of Lipoid Percentages in Control and Gaucher Organs

Description, Clinical and Pathologic	Fresh Tissue							Formaldehyd Tissue				Gaucher Tissue Fixed in Liquor Formaldehydi				
	Man, Age 40 yrs. Marked Fatty Degeneration of Viscera (Tuberculosis)	Child, Age 2 yrs. Meningitis	Child, Age 7 yrs. Scarlet Fever	Child, Age 11 mos. Meningitis, Aut. 1558	Child, Age 1 yr. Pneumonia, Aut. 1563	Average	Average Percentage of Total Lipins	Aut. 1558	Aut. 1563	Average	Average Percentage of Total Lipins	First Determination	Second Determination	Third Determination	Average	Average Percentage of Total Lipins
Spleen:																
Acetone-insoluble ppt. (Lecithin)	1.97	2.9	3.45	1.33	2.17	2.32	11.2	1.46	2.32	1.89	8.8	14.3	8.43	11.28	11.33	20.7
Cholesterol and phosphatids	15.13	24.3	12.47	12.4*	16.3	16.08	78.	18.66	14.17	16.4	77.6	13.2*	41.6	42.4	42.	77.7
Fat (fixed)	4.5	.3	.52	.93	3.05	1.86	9.	1.5	4.77	3.14	14.6	.26	?	1.2	.73	1.3
Total Lipins	21.9	27.5*	16.44	14.66	21.42	20.51	.....	21.62	21.26	21.44	.....	27.57*	50.03+	54.8	54.06	
Liver:																
Acetone-insoluble ppt. (Lecithin)	1.64	2.5	.....	2.01	3.5	2.66	11.5	2.48	2.11	2.28	8.2	10.11	11.21	.....	10.66	28.6
Cholesterol and phosphatids	19.8	13.2	.....	18.4	12.4*	15.95	69.0	22.5	21.4	21.95	79.	23.72*	27.07	.....	25.49	68.2
Fat (fixed)	9.8	1.08	.....	2.74	?	4.37	19.	2.82	4.2	3.51	12.2	.09	?.	.....	1.04	2.8
Total Lipins	31.2	17.73	.....	23.15	15.9+	22.99	.....	27.8	27.71	27.77	.....	33.9+	40.48	.....	37.19	

* Some lost.

maldehyd material uncertain. A positive result would be of much more value than a negative one, and the absence of anisotropic droplets, for example, in most of the cells may be interpreted either as indicating an alteration produced by liquor formaldehydi or the presence of other lipoid bodies that are isotropic. It may be for this reason that Lutz[11] found the Lorain-Smith-Dietrich stain for lipoids in the formaldehyd-fixed material of Risel's and of Schlagenhaufer's cases negative, whereas Schultze,[9] who apparently examined the material about two years earlier, when it was fresher, found the reaction positive. The microchemical reactions in our case are practically identical with those described by Lutz[11] in his first case and very similar to those of

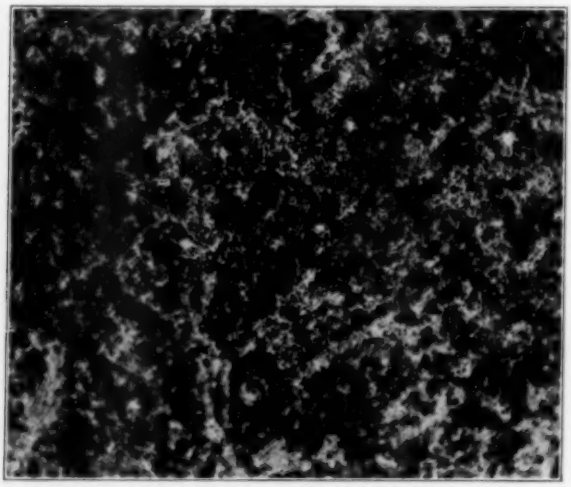

Fig. 4.—Photomicrograph of a preparation, × 85, stained for lipoids after Lorain-Smith-Dietrich's method. The distinctive large pale cells are more or less filled with lipoid substances and stand out as irregular round black masses.

Schultze.[9] Both of these authors examined the material of the Risel and Schlagenhaufer cases and concurred in the identity of the cells and the lipoid character of the intracellular droplets of their cases and the cases of Gaucher's disease. Sapegno[12] also noted the same lipoid infiltration in the cells of his case of Gaucher's disease, but asserted that the cells were of lymphocytic origin and not endothelial and reticular as the other authors contended.

There is no question about the identity of the cells and the lipoid character of the material accumulated in our cases with the foregoing cases of Gaucher's disease and of "grosszellige Hyperplasie der Milz."

This does not imply that the lipoids are identical. They are so complicated and difficult to separate that conflicting results with the crude microchemical reactions are not at all surprising, and no attempt to separate these cases on the basis of slight variations in their reactions to our present unreliable microchemical reactions would be warranted.

The quantitative determination of the lipoids is equally difficult and uncertain (Bang[26]), but for our purposes it serves in a general way to confirm the conclusions, based on the microchemical reactions, that there is a great increase in lipoids with the Gaucher liver and spleen and that lipoids, such as lecithin-like bodies as well as cholesterol compounds, are mainly responsible. In this way the question as to whether the negative reaction of most of the large cells to cholesterol is due to alterations produced by formaldehyd fixation or to the presence of different lipoid bodies is definitely determined. We have not found any complete quantitative analysis of the different lipoids and fats in the spleen. Wells[27] shows that the dried normal spleen contains 14.2 per cent. lipins, a figure that is somewhat lower than our determinations for the control spleens; though in one case (Table 3) the figure of 14.66 per cent. was very close. Our higher average is probably due to our material being mostly pathologic. In the normal liver[27] the lipin content amounts to 5 per cent. of the moist weight or about 22 per cent. of the dried weight; but in pathologic organs the lipins may run up as high as 75 per cent. of the dried weight. According to Rumpf,[28] the fat (total lipin) content of the liver varies from 2 to 56 per cent. of the dried weight with an average of 19.6 per cent., and the content of the spleen varies from 1.4 to 24.8 per cent., with an average of 9.96 per cent. His figures for the liver correspond closely with our determinations, but those for the spleen are much lower. No determination of the different lipoids was available. Aschoff[17] showed that cholesterol formed 5.9 per cent. of the normal liver cell. Our determinations give a general conception of the distribution and relation of the main lipoids and fats in the average organ obtained at necropsy, but the number of cases analyzed is too few to make the results very reliable. The findings would also indicate that liquor formaldehydi does not interfere so extensively with the extractibility of lipoids and fats, but that a good working conception may be obtained of their amount and should encourage similar determinations to be made on this and other rare and interesting xanthelasmic tissues, of which usually nothing but formaldehyd-fixed material is available. From the microchemical reac-

27. Wells, H. Gideon: Chemical Pathology, Philadelphia, W. B. Saunders & Company, 1914, p. 495.

28. Rumpf: Ueber den Fettgehalt des Blutes und einigen Organe des Menschen, Virchows Archiv. f. path. Anat., 1903, clxxiv, 163.

tions it is very probable that the lipoid substances, especially a lecithin-like body and cholesterol that are quantitatively greatly increased in the liver and spleen as a whole, are accumulated in the droplets within the large pale cells.

The nature of the process which underlies this peculiar accumulation of lipoids opens up a broad field for speculation. The individual large pale cells in Gaucher's disease are not specific for this disease. They occur not only in Gaucher's disease and in allied conditions, such as Schultze's case of lipoidemia, but also in various more common pathologic lesions such as chronic granulation tissue[2] and chronic pyometra and pyosalpingitis. They have even been noted free in the

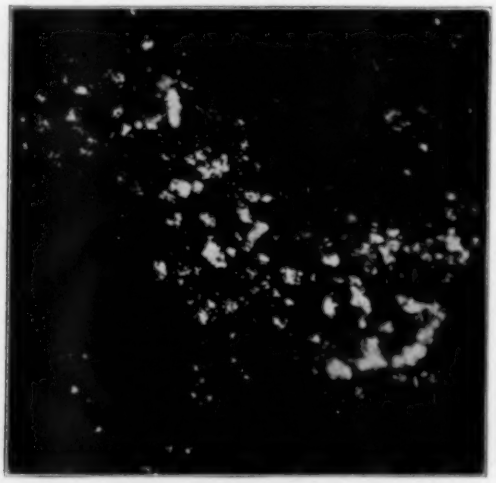

Fig. 5.—Photomicrograph of a frozen section, × 160, through a polarizing microscope showing the doubly refractile liquid crystals in many of the large pale cells. Note that this anisotropic material is much more abundant is some cells than in others.

blood stream (Kawamura[29]). Whether or not they occur in the blood in Gaucher's disease has not been reported, largely, perhaps, because no careful search was made for them. They do not necessarily represent a degenerative or a pathologic process, for Ciaccio[30] and Kusunoki[4] have noted them in normal conditions, the former as "Lecithin-

29. Kawamura: Cholesterinesterverfettung, Jena, Fischer, 1911.

30. Ciaccio, Carmelo, Beitrag zum Studium der Zelllipoide in normalen und pathologischen Verhaltnissen und einer besonderen Entartung von lipoid Typus (Lecithinische Entartung), Zentralbl. f. allg. Pathol. u. path. Anat., 1909, xx, 771.

zellen" in the blood-forming tissues, and the latter as "lipoid" cells in the spleen. They both point out that they have the physiologic functions of absorbing and disposing of lipoid substances in the tissues, and that in pathologic conditions these cells may accumulate in great numbers. Chalatow[24] termed this accumulation of lipoid material "xanthomatosis" or "myelinosis," according as the material accumulated in the supporting tissue of the organs or in the parenchyma, the former being an active process, the latter a passive one. Accordingly, he would call the spleen described by Lutz a case of xanthomatosis. Kusunoki[4] maintained that the lipoid cells were mainly reticular cells, though some were derived from the endothelium of the lymph sinuses, and had the same origin as the pigmented cells of the spleen. Anitschkow[14] held that these lipoids were also present in the reticulo-endothelial cells of the bone marrow and lymph nodes and in the Kupfer cells of the liver, and that they took up carmin and other vital stains and iron pigment. He maintained that in his experimental rabbits the accumulation of cholesterol involved a certain system of cells, that is, the reticulo-endothelial cells of the spleen, bone marrow, lymph glands and the Kupfer cells of the liver, and suggested the analogy with Gaucher's disease, but refrained from asserting any identity between the two processes. Soper[31] pointed out recently that the spleen owes its part in cholesterin metabolism mainly to the large proportion of the reticulo-endothelial system of the body (designated by Landau[32] and Aschoff as the "endothelial Stoffwechsel Apparate or Milzapparate," which the latter hold plays the main rôle outside of the liver in the absorption, transformation and retention of cholesterin) which it occupies. It is this system which forms the lipoid cells, which takes up the vital stains, which is concerned in the iron pigment metabolism and which is the seat of the striking changes in experimental cholesterin-steatosis following a prolonged cholesterol diet. Of the various conditions in which the lipoid cells accumulate in large numbers, it is only in the experimental type produced in rabbits by excessive cholesterol feeding that one is justified in considering the lipoid material as cholesterol to the exclusion of other lipoids and, even here, quantitative determinations should be made to confirm the more or less inadequate microchemical methods of recognizing the main lipoid bodies. It is because of the inadequacy of microchemical methods and the close relation of one lipoid to another that, in the cases of human xanthelasmic conditions, it would be hazardous to limit the lipoids to one par-

31. Soper, W. B.: Ueber Beziehungen der Milz zum Cholesterin-stoffwechsel, Beitr. z. path. Anat. u. z. allg. Path., 1915, lx, 233.

32. Landau, M., and McNee, J. W.: Zur Physiologie des Cholesterin-stoffwechsels, Beitr. z. path. Anat. u. z. allg. Path., 1914, lviii, 667.

ticular type. Accordingly, we hesitate to speak of cholesterin infiltration, "cholesterinverfettung," etc., and prefer the more general term "lipoid" until more is known about lipoids and their recognition microchemically.

In our case it is important to recall that the medulla of both suprarenal glands was almost replaced by the large pale cells similar to those in the spleen and lymph nodes, a fact that is difficult to explain. According to Krylow[33] the suprarenal cortex regulates the lipoid metabolism, and its inefficiency would cause the accumulation of local deposits of lipoid in other organs. No such deposit has hitherto been noted in the suprarenal medulla. Possibly, this is because the other cells were filled with lipoid and the accumulation still continued, resulting in a lipoid infiltration of the reticulo-endothelium of the medulla. But for this medullary change our case, histologically, seems to be a human counterpart of Anitschkow's experimental cholesterinsteatosis. A more serious objection to this assumption of similarity is in the fact that both microchemically and quantitatively cholesterin was not the only lipoid present; in fact, it was relatively less abundant than a lecithin-like body.

The relation of our case to Gaucher's disease is a very important consideration, inasmuch as it deviates in some respects from the cases that have been reported. The absence of any knowledge of the underlying cause of the lesions in Gaucher's disease, the paucity of the cases that have been thoroughly described, and the lack of unanimity of opinion among the different authors regarding the nature and origin of the large pale cells, some holding that they are endothelial cells (Bovaird,[34] Brill[35]), others that they are changed reticular or both reticular and endothelial cells (Risel,[7] Schlagenhaufer[36]) and still others maintaining that they are derived from embryonic lymphoid cells (Mandelbaum,[37] Sapegno[12]) makes it difficult if not impossible to delineate what is the typical histologic picture of Gaucher's disease. The differences of opinion are probably due to variation in the reaction of the cells to the underlying processes, resulting in the predominance of different cells in the individual cases. Our case is the first one noted

33. Krylow, D. D.: Experimentelle Studien über Nebennierenrinde, Beitr. z. path. Anat. u. z. allg. Path., 1914, lviii, 434, 474.

34. Bovaird, D.: Primary Splenomegaly, Am. Jour. Med. Sc., 1900, cxx.

35. Brill, N. E.: Mandelbaum, F. S., and Libman, E.: Primary Splenomegaly, Gaucher Type, Am. Jour. Med. Sc., 1905, cxxix, 491.

36. Schlagenhaufer, F.: Ueber mist familiar vorkommende, histologisch charakteristische Splenomegalieen (Typus Gaucher: (Eine Systemerkrankung des lymphatisch-hämopoetischen Apparates), Virchows Arch. f. path. Anat., 1907, clxxxvii, 125.

37. Mandelbaum, F. S.: The Pathology of Primary Splenomegaly, Jour. Exper. Med., 1912, xvi, 797.

in an infant, and the more diffuse character of its lesions may well be the manifestation in an infant of the same process underlying Gaucher's disease. In this case the medulla of the suprarenal glands and the adventitia of some of the vessels showed the same accumulation of lipoid cells. The theory that Gaucher's disease involves only the hematopoietic organs is not entirely substantiated by the cases reported. In Risel's case a cluster of the large pale cells was noticed in the thyroid. Moreover, in most of the cases large numbers of the cells were described in Glisson's capsule of the liver as well as in the sinusoids, a fact that is opposed to the strictly hematopoietic nature of the disease. The only part of the liver that enters into blood formation during fetal life is the sinusoids. The explanation that the large lipoid cells are transported to the liver from the spleen by the blood stream is untenable, inasmuch as no such cells have ever been seen in the blood stream or in the portal vessels. It is much more probable that Gaucher's disease represents a "Systemerkrankung" not of the hematopoietic organs as such but of the reticulo-endothelial cells of the body, and most strikingly manifested in the hematopoietic organs, because of the prominence of the reticulo-endothelial cells in them. This conception would explain our case much more satisfactorily and also the cases of Gaucher's disease reported by the German authors. The streaking of the cytoplasm of the large pale cells, which was noted by Marchand[6] and Mandelbaum[12] was considered a characteristic of the cells in Gaucher's disease, but it has not been noted in the reports of most of the cases and is not peculiar to Gaucher's disease, inasmuch as Pick has shown that it occurs in the lipoid cells in old chronic granulation tissue of cases of chronic salpingitis,[1] and that it is due to the shrinkage of the cytoplasm following the solution of clusters of needle-like lipoid crystals. Consequently, this streaking indicates the presence of a cholesterin compound, but it is of minor anatomic importance and should play little part in the interpretation of the process as a whole, and its absence in our case does not mean that our case is not one of Gaucher's disease. The character of the large pale cells of our case and of the lipoid within them is identical with that of the cases of "grosszellige Hyperplasie" described by Schultze and Lutz and, based on the statements of these authors that the cells in their cases were identical with the cases of Gaucher's disease reported by Risel, Schlagenhaufer and DeJong and Heukelom, and on the involvement of essentially the same tissues, we feel that we are fully justified in classing our case as an example of Gaucher's disease rather than reporting it as a unique and hitherto undescribed pathologic process.

The peculiar accumulations of lipoid cells, in Gaucher's disease, in cases of lipoidemia and in various subcutaneous tissues represent vary-

ing degrees of involvement of the same general system, that is, the reticulo-endothelial system (including the stellate cells of the liver) which controls the normal disposition of the lipoids of the body.[14] Such accumulations are designated xanthelasmic diseases, and indicate a disturbance of lipoid metabolism. The latter may be so seriously involved that the cells which normally dispose of the lipoids are unable to do so, resulting in a lipoid infiltration of other cells, such as the parenchyma of the liver, a condition designated as myelinosis (Chalatow[24]). The causes underlying this disturbance of the metabolism are obscure, but Anitschkow's[38] work in producing local accumulations in subcutaneous tissue by inducing a general lipoidemia, from cholesterin feeding in rabbits and a local injury to the subcutaneous tissue, is very suggestive. It may well be that in these accumulations in man there is a predisposing susceptibility of the reticulo-endothedlial system due to a disturbance in the lipoid metabolism which may be manifested by a lipoidemia, and it only requires some toxic, or other injury of a part or of all of the system, to lead to a local or a general more diffused accumulation of lipoid substances in this system. The factors that underlie the disturbances in lipoid metabolism and the accumulation of lipoid cells are unknown, but undoubtedly vary in the different cases, being more deep-seated and inherent in the body economy in the more diffused and extensive cases, such as in our case. In accordance with this conception, in Schultze's case of lipoidemia, accumulation of the lipoid cells in the spleen represents a local involvement of the reticulo-endothelial system, the average case of Gaucher's disease, a more diffuse involvement of the same system, while our case of this disease represents an even more serious involvement, the accumulation of the cells occurring in the reticulo-endothelium of the suprarenal medulla and of the intestinal tract as well as in other tissue cells. The lipoid substance is not necessarily the same in each case, for undoubtedly various combinations of lipoids occur with one predominating in one case, and another in other instances. While our case resembles in some respects Anitschkow's experimental cholesterinsteatosis, it is not an example of "Cholesterinesterverfettung," inasmuch as both our microchemical and quantitative studies show that a lecithin-like body is the chief lipoid present with also a prominent cholesterol body.

CONCLUSIONS

1. In Gaucher's disease the liver and the spleen show not only a marked increase in the lipin content, but also a serious alteration in normal relations of the lipins to each other. The fixed fats are greatly

38. Anitschkow: Ueber experimentelle erzeugte Ablagerungen von Cholesterinester und Anhaufungen von Xanthomzellen in subcutanen Bindegewebe des Kaninchens, München. med. Wchnschr., 1913, No. 46.

reduced, while the lipoids, such as lecithin and cholesterol, are greatly increased. In our case a lecithin-like body predominated, but a cholesterin compound may prevail in other cases.

2. In Gaucher's disease, lipoid substances accumulate in the form of small droplets within the cytoplasm of the tissue cells, resulting in the formation and accumulation of the distinctive large pale cells so characteristic, histologically, of this disease.

3. Gaucher's disease is due to a disturbance of lipoid and fat metabolism, resulting in the accumulation of lipoid substances in the cytoplasm of the large pale cells that are mostly transformed reticulo-endothelial cells of the spleen, lymph nodes and bone marrow and the stellate cells of the liver. These cells have the physiologic property of disposing of the fats and lipoids, and comprise the "endothelial Stoffwechselapparate." It is thus a system disease, but involves the hematopoietic organs only secondarily in that they are very rich in the reticulo-endothelial cells.

4. Those organs that contain the reticulo-endothelial cells in large abundance (spleen, lymph glands, bone marrow, liver, stellate cells of Kupfer, etc.) show the most changes; but specific parenchymal cells may absorb some of the lipoid in a very advanced case.

5. Gaucher's disease belongs to the group of xanthelasmic conditions which are characterized by a more or less diffuse accumulation of lipoids in reticulo-endothelial or in fibroblastic cells in one or more organs. It represents a more diffuse and widespread involvement of the "Endothelial Stoffwechselapparate" than those cases of "grosszellige Hyperplasie der Milz" in diabetic lipoidemia, with an underlying cause that is more deepseated and inherent in the body economy.

We wish to express our thanks to Dr. J. H. Mason Knox, Jr., of Baltimore, for his courtesy in allowing us to use his material; to Dr. Paul J. Hanzlik for many valuable suggestions for our chemical study, especially in the evolution of our method for extracting the fats and lipoids, and to Prof. Howard T. Karsner for many suggestions and encouragement in carrying out our studies.

THE CONTROL OF EXPERIMENTAL CRETINISM *

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The work reported in this paper was undertaken at the suggestion of A. J. Carlson, primarily for establishing a biological test for the active principle of the thyroid gland in the blood.

A few years ago Hunt,[1] by means of the acetonitril test, thought that he had a reliable test for the active principle of the thyroid in the blood. Later investigators (Carlson and Woelfel,[2] Lussky[3]) failed to detect any active principle of the thyroid in the blood of the dog, and in rabbits and guinea-pigs in experimental hyperthyroidism by means of this test. Hunt studied the blood of clinical cases of exophthalmic goiter and obtained a positive test in two out of three cases. On the other hand, Carlson and Woelfel could not get a positive reaction in a case studied by them. Carlson also tested his own blood after having taken thyroid until toxic symptoms arose, with negative results. The presence of iodin in the blood in "hyperthyroidism" has recently been shown by Jones and Tatum.[4] They found that intravenous injections of blood serum from hyperthyroid-fed rabbits increased the iodin content of the thyroid glands of the rabbits which were injected.

Another method of attack relates to the changes of excitability of the depressor nerve. Von Cyon and Oswald[5] found that injection of thyroid extract increases the irritability of the depressor nerve. Asher and Flack reported some interesting results on the excitability of the depressor nerve before and after stimulation of the thyroid nerves, and the effects of small intravenous injections of epinephrin before

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* From the Hull Physiological Laboratories of the University of Chicago.

1. Hunt, Reid: (a) Influence of Thyroid Feeding and of Various Foods upon Poisoning by Acetonitril, Proc. Soc. Exper. Biol., N. Y., 1905; Jour. Am. Med. Assn., 1907, xlix, 1323; (b) The Probable Demonstration of Thyroid Secretion in the Blood in Exophthalmic Goiter, Jour. Am. Med. Assn., 1907, xlix, 240.

2. Carlson, A. J., and Woelfel, A.: On the Internal Secretion of the Thyroid, Am. Jour. Physiol., 1910, xxvi, 32.

3. Lussky, H. O.: Further Studies of the Acetonitril Test for Thyroid Substance in the Blood, Am. Jour. Phiysiol., 1912, xxx, 63.

4. Jones, A. P., and Tatum, A. L.: On the Demonstration of the Variations in the Thyroid Colloid in Conditions of Hyperthyroidism and Hypothyroidism, THE ARCHIVES INT. MED., 1913, xii, 225.

5. von Cyon, E., and Oswald, A.: Ueber die physiologischen Wirkungen einiger aus der Schilddruse gewonnener Producte, Pfluger's Arch. f. d. ges. Physiol., 1901, lxxxiii, 199.

and after such stimulation. The excitability of the depressor nerves is increased during or immediately following the stimulation of the thyroid nerves. According to Asher and Flack,[6] the animals are also sensitized to epinephrin through stimulation of the thyroid nerves. They ascribe these results to the action of increased quantity of thyroid secretion poured into the blood on stimulation of the thyroid nerves. After extirpation of the thyroid gland, stimulation of the thyroid nerves failed to produce increased excitability of the depressor nerve. According to their report, intravenous injection of thyroid extract acts similarly to stimulation of the thyroid nerves. Ossakin[7] (1914) working in Asher's laboratory repeated and apparently confirmed Asher and Flack's work.

The experiments were carried out on cretin rabbits produced by complete thyroidectomy. Five lines of investigation were undertaken. 1. The transfusion of normal blood serum. 2. The transfusion of "hyperthyroid" blood serum. 3. The feeding of Standard U. S. P. thyroid products. 4. The feeding of Koch's thyroid metaprotein. 5. The feeding of Kendall's Extract B.

I. PRODUCTION OF CRETINS

The litters of rabbits were all raised from healthy albino stock of approximately uniform size. They were all kept in cages with their mother until 6 weeks of age, after which they were separated and the mother was again mated.

The animals were fed with oats, carrots, alfalfa hay and water daily. It is essential that the diet of the different litters be as uniform as possible, because of the great variation of growth curves with various foods. In this way the growth curves of various litters were made practically uniform.

Thyroidectomy was performed on the young rabbits at the age of 2 to 3 weeks, when their average weight was about 175 gm. One or two members of each litter were kept as controls. In the removal of the thyroids, the method of Hofmeister was followed, with usual aseptic procedures. Through a median incision of about 2 cm. over the prominence of the larynx, the subcutaneous tissue and muscles were separated, bringing the thyroid cartilage into view. The thyroid glands lying on both sides of the trachea are, in rabbits, always connected at the lower pole by an isthmus, more or less flat and membranous. The gland is usually so closely adherent to the thyroid cartilage,

6. von Asher, L., and Flack, M.: Die innere Sekretion der Schilddruse und die Bildung des inneren Sekretes unter dem Einflusz von Nervenreizung. Ztschr. f. Biol., 1911, lv, 83.

7. Ossakin, N.: Zur Frage des Innervation des Glandula Thyroidea, Ztschr. f. Biol., 1914, lxiii, 444.

especially at its posterior end, that it can be separated only with difficulty. With a fine pointed probe, the lower pole is carefully separated from the recurrent laryngeal nerve and the surrounding pericapsular tissue. It is essential that the recurrent laryngeal nerve should not be injured because of the fatal dyspnea following such an injury. Care was also taken to leave the external parathyroids intact. One or more may usually be seen in the pericapsular tissue. The arteries were either ligated before division or, if small, they were clamped and crushed with forceps before division. The thyroid tissue was carefully dissected away from the thyroid cartilage and this lobe, together with the connecting isthmus, was reflected over the remaining lobe. This lobe was separated in a similar way.

Great care has to be taken to remove all of the thyroid tissue, because it is surprising how fast small remnants of tissue will hypertrophy. Such remnants, if present, on postmortem examination were usually found on the posterior end, closely adherent to the thyroid cartilage. These nodules always showed marked evidence of hypertrophy. However, these remnants were never more than one-tenth of the size of one of the lobes. Out of over 140 operations I have produced typical symptoms of cretinism in eighty-six cases. There is also some variation in the different litters in the location of the gland with respect to the thyroid cartilage. In some litters, on account of the difficulty of removal it was impossible to get cretins in more than 25 per cent. of cases. On the other hand, the perfect ease with which the thyroid gland could be removed in other cases was shown by the development of symptoms of cretinism in 90 per cent. after operation. Experimental cretinism in rabbits has been studied by Moussu,[8] Hofmeister,[9] Haushalter and Jeandelize,[10] Leonhardt,[11] Blumreich and Jacoby,[12] and Tatum.[13]

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8. Moussu: Effets de la thyroidectomie chez nos animaux domestiques, Compt. rend. Soc. de biol., 1892, xliv, 271.

9. Hofmeister, F.: (a) Experimentelle Untersuchungen über die Folgen des Schilddrüsen Verlustes, Beitr. z. klin. Chir., 1893-94, xi, 441; (b) Zur Frage nach den Folgeguständen bei Schilddrüsenexstirpation, Deutsch. med. Wchnschr., 1896, xxii, 354; (c) Uber Störungen des Knochenwachstums bei Kretinismus, Fortschr. der Röntgenstr., 1897-1898, 1.

10. Haushalter, P., and Jeandelize, P.: Retard de developpement et etat cretinoide a la suite de la thyroidactomie chez un agneau et chez un lapereau, Compt. rend. Soc. de biol., 1902, liv, 597.

11. Leonhardt, M.: Experimentelle Untersuchungen über die Bedeutungen der Schilddrüse für des Wachstum im Organismus, Virchow's Arch. f. path. Anat., 1897, cxlix, 341.

12. Blumreich, S., and Jacoby, M.: Experimentale Untersuchung über die Bedeutung der Schilddrüse und ihrer Nebendrusen für den Organismus, Pflüger's Archiv. f. d. ges. Physiol., 1896, lxiv, 1.

13. Tatum, A. L.: Morphological Studies in Experimental Cretinism, Jour. Exper. Med., 1913, xvii, 636.

In the present series the onset and development of the cretin symptoms were always similar. About two weeks after the operation the hair becomes noticeably drier and does not lie smooth and flat on the skin as normally. It stands up and can be pulled out very easily. At the same time there is a gradual retardation of growth. This is noticeable as early as the third week after the operation. This retardation of growth is greatest from the eighth to the twelfth week, as shown in Figure 1. By the tenth week, the average weight of the cretins is 750 gm., while the normal controls weigh approximately 1,400 gm. From this time the growth curve of the cretins deviates even more rapidly from that of the normal, but the growing period is longer in the case of the cretins. This was true in all cases under observation.

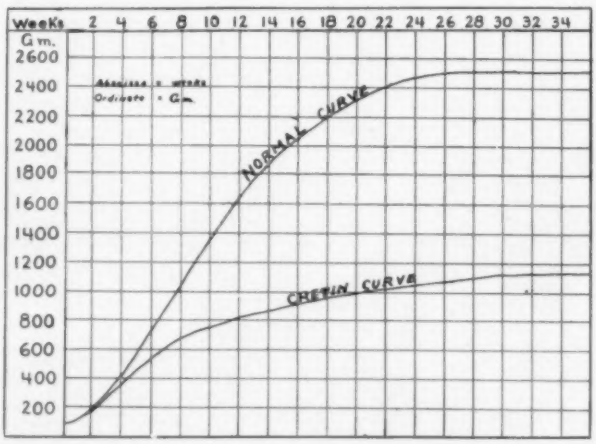

Fig. 1.—The growth curves of four normal control and three cretin rabbits.

Figure 2 shows two typical cretins with normal control at the age of 12 weeks. The weight of the normal rabbit is 1,630 gm., while the weights of the cretins are 760 and 840 gm., respectively. The posture of the cretin is typical. The limbs are short and the muscles are too weak for support. The bones show a pseudorickety condition which Hofmeister[9] calls "chondrodystrophia thyreopriva." The hair becomes coarse and can be pulled out in bunches. The skin becomes dry, thick and scaly, gradually turning into a typical eczema covered with crusty scales. This is always most marked about the head, ears, shoulders and legs. The proportionate growth of the abdomen over that of the rest of the body gradually increases and the cretin acquires the descriptive "pot belly" type of abdomen. They are slow, awkward and move about very reluctantly. If not disturbed, they may remain for hours

in the same place. Haushalter and Jeandelize[10] speak of a swollen, myxomatous condition of the skin which developed gradually in all of their cretins. There was no typical myxedema noticed in any of my cretins. It is evident that myxedema so characteristic of post-operative thyreopriva in man, is not an accompanying symptom of cretinism in rabbits. The chronic, progressive fatal cachexia described by Hofmeister, was not apparent in my cretins. I have kept sixteen cretins alive for over a year without any evidence of cachexia as described by Hofmeister. Of course, there was a marked muscular weakness, especially noticeable in the limbs, but the animals always appeared to be in a fairly well nourished condition.

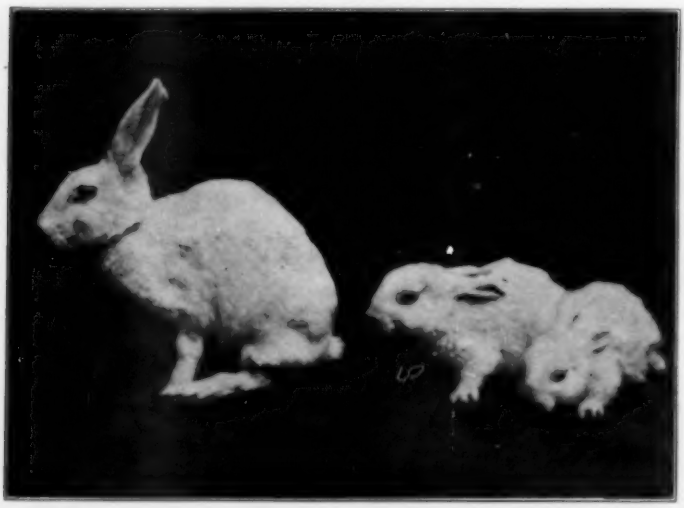

Fig. 2.—Normal control rabbit and two cretins from same litter. Age 12 weeks. Weight of normal, 1,630 gm.; cretins, 840 and 760 gm.

II. BLOOD TRANSFUSION

(*a*) *Normal Blood Serum.*—The blood serum used for transfusion was taken from rabbits from the same stock as those used for experiments. Sixteen normal, healthy grown rabbits were kept for bleeding purposes. These were bled alternately, and never oftener than once every three weeks, for the purpose of keeping the animals in the best possible condition and for giving the thyroid gland a chance to replenish its secretion, assuming that it has an internal secretion. The greatest possible aseptic precautions were taken in all these experiments. The animal was bled 25 c.c. from the ear, the blood defibrinated,

filtered at once, rapidly centrifuged and the serum injected intravenously into the cretin rabbits. Four cretins averaging 350 gm. were used for transfusion. Injections were made into the ear veins, under the usual aseptic precautions, with a fine needle. The amount injected in the four cases varied from the beginning of the experiment, the smallest being 2 c.c. and the largest 5 c.c. These transfusions were made four times weekly. There was no evidence of any toxicity, so these doses were gradually increased. Toward the end of the experiment, the amount injected in the four cases ranged from 6 c.c. to 10 c.c. Figure 3 represents the average growth of the controls and of the cretin rabbits which have been transfused. The normal cretin curve is identical with the lower curve. Suffice it to say, that these curves

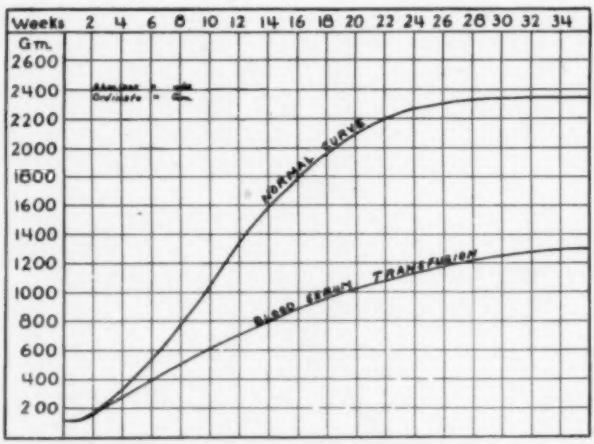

Fig. 3.—The growth curves of three normal controls and four cretin rabbits which had been transfused with normal blood serum.

differ in no respect from those of Figure 1, except that the maximum growth of control, normal cretin and transfused cretin are somewhat less.

Figure 4 shows a control rabbit with two cretins at the age of 16 weeks after transfusion of the cretin for two and one-half months. The weight of the control was 1,850 gm., and that of the cretins 890 and 950 gm., respectively. There is no evidence of any improvement due to the transfusion. They are essentially cretins differing in no respect from the normal cretins shown on the other pictures.

(*b*) *"Hyperthyroid" Blood Serum.*—Fifteen normal, healthy, grown rabbits were kept in separate cages as in the preceding experiment. These were fed increasing doses of Standard U. S. P. thyroid

four times per week, until symptoms of toxicity resulted. This overdose of thyroid, or "hyperthyroidism," was kept up throughout the experiment. The average toxic dose for these rabbits was 0.65 gm. The animals were bled alternately as in the preceding experiments. The procedures, as bleeding, centrifuging and transfusions were similar. The same general precautions were taken to have the transfusion

Fig. 4.—Normal control and two cretin rabbits which were transfused with normal blood serum. Age, 16 weeks. Weight of normal, 1,850 gm.; cretins, 890 and 950 gm.

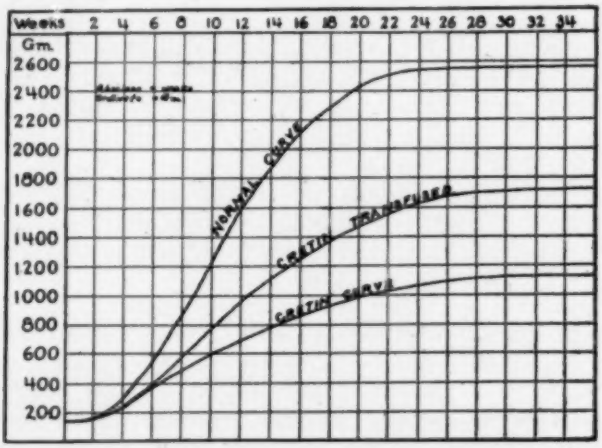

Fig. 5.—The growth curves of four normal controls, two cretins, and six cretins which had been transfused with "hyperthyroid" blood serum.

done under as nearly sterile conditions as possible. Six cretin rabbits from four litters, containing eight cretins and four normal controls, were used for transfusion. Injections were begun on the fourth week with amounts varying from 3 to 5 c.c. These transfusions were made four times a week. Cretin rabbits weighing 350 gm. will not tolerate

more than 5 c.c. because of the apparent toxicity of this serum. These doses were gradually increased as in the preceding experiments. By the twentieth week, the amount injected varied from 8 to 12 c.c. in the different rabbits.

The growth curves are shown in Figure 5. The upper curve represents the growth of normal, the lower, the normal cretin, and the middle, the growth curve of the cretin rabbits which had been transfused with the "hyperthyroid" blood serum. The upper and lower curves are similar to the curves of preceding figures. The middle curve is typical of the effects of transfusion of the "hyperthyroid" blood serum. During the first two weeks, there is no change from the normal cretin curve, after which there is a gradual increase. This increase, however, is not so marked as in the cretins which had been fed thyroid. The average maximum growth attained in the six cretins which were injected, was 1,750 gm. The average weight of the normal

Fig. 6.—Normal control and two cretin rabbits from same litter. Age, 28 weeks. Transfused with "hyperthyroid" blood serum. Weight of control, 2,350 gm.; cretins, 1,650 and 1,720 gm.

was 2,550 gm. and that of cretins, not transfused, 1,125 gm. This gives an average increase in weight of transfused rabbit of 600 gm. over that of normal cretins.

Figure 6 shows two transfused cretins and one control at the age of 28 weeks (7 months). The weight of the control is 2,550 gm. and of transfused, 1,650 and 1,720 gm., respectively. The hair is abundant, smooth and lays flat. The skin shows no signs of any scaly or eczematous condition. The weakness of muscles, bones and joints has disappeared. The animals appear as alert and active as the normal rabbits.

III. THYROID FEEDING

The clinical literature on the beneficial effects of thyroid feeding on cretinism seems conclusive. In man, however, it is frequently difficult to decide whether we are dealing with cases of pure cretinism due to deficiency of thyroid activity, or with other diseases.

Kutschera,[14] who had more than one thousand cases of cretins under observation, claims that it is often next to impossible to differentiate cretinism from certain forms of rachitis. Thyroid medication on these patients was carried on by Kutschera over a period of two years, with complete cure in 42.8 per cent. of the cases, decided improvement in 48.6 per cent., and in 8.6 per cent. there was no evidence of any improvement. The hair became normal, myxedema disappeared and bone deformities decreased. Kutschera plotted many growth curves, showing that in 377 cases the increase of growth was above that of the normal. Many clinicians and investigators, however, could not bring the growth of cretins up to that of the normal individual. Kutschera says that the increase of growth can be taken as the best criterion of the value of thyroid therapy.

Pick and Pineles,[15] in 1910, reported their results on the activity of various laboratory preparations in experimental cretinism in young goats. With the desiccated thyroid there was a rapid improvement. A thyroidin preparation was negative in activity. On the other hand, earlier investigators (Baumann,[16] Ewald,[17] Lichtenstern, Magnus Levy[18]) claim that thyroidin is just as active in removing the symptoms of thyroid deficiency as the desiccated thyroid. The results of the earlier investigators, however, are of little value because their work was mostly confined to dogs which died early of parathyroid tetany, due to removal of the parathyroids with the thyroids. According to Pick and Pineles, the symptoms of cretinism can be controlled by feeding thyreoglobulin. Oswald[19] and Magnus Levy[18] also found that thyreoglobulin is active. Moreover, Pick and Pineles[15] found that pepsin and trypsin split products are still effective when digestion is not allowed to go on for a long time. Long continued digestion will entirely destroy the thyroid activity. If Hunt's acetonitril

14. Kutschera, V.: (a) Das Groszenwachstum bei Schilddrüsenbehandlung des endemischen Kretinismus, Wein. klin. Wchnschr., 1909, xxii, 771; (b) Der endemische Kretinismus, seine Ursachen, und seine Behandlung, Wien, Alfred Holder, 1911.

15. Pick, E. P., and Pineles, F.: Untersuchungen über die Physiologisch Wirksame Substanz der Schilddrüse, Ztschr. f. exper. Path. u. Therap., 1909, vii, 518.

16. Baumann, E.: Ueber die Wirksamkeit des Thyroidins, München. med. Wchnschr., 1896, xliii, 476.

17. Ewald, J. R., and Rockwell: Exstirpation der Thyreoidea an Tauben, Pflüger's Arch. f. d. ges. Physiol., 1890, xlvii, 160.

18. Magnus-Levy: (a) Uber Myxodem, Ztschr. f. klin. Med., 1904, lii, 201; (b) Organtherapie und innere Sekretion, Mod. ärtztl. Bibl., Berlin, 1906; (c) Die Therapie d. Gegenw., 1907.

19. Oswald, A.: (a) Die Schilddrüse und ihr Wirksames Principel, Biochem. Centralbl., 1903, i, 249; (b) Die Eiweiszkörper der Schilddrüse, Ztschr. f physiol. Chem., 1899, xxvii, 14.

test is to be taken as a reliable method of judging the activity of a given thyroid preparation, then the above results agree with those of Koch,[20] who determined the activity of different split products of thyroid by means of the acetonitril test. He found that the thyreoglobulins and metaprotein fractions are more active than the original product. The other products of thyroid hydrolysis, iodothyrin, primary and secondary albumose, show a marked decrease of activity. Lower split products are entirely inactive.

Recently Kendall[21] reported some interesting results on certain split products of the thyroid gland which he calls "Extract A" and "Extract B." Extract A affects the nitrogen metabolism, body weight and temperature. It causes marked tachycardia, nervousness and tremors. Extract B shows no toxicity. The skin and hair become entirely normal in human cretins. The weakness of muscles, bones and joints soon disappear. Schaefer[22] found that the addition of small amounts of thyroid tissue to the normal diet of white rats causes a great increase of food consumption with acceleration of the growth curve. Recently, Gudernatsch[23] fed fresh thyroid tissue to young tadpoles and found that this tissue has the power to excite differentiation, but it lacks the power to produce growth. On the other hand, he claims that thymus and spleen cause growth without differentiation. It is evident, however, that since the thyroid tissue is rather toxic, as Gudernatsch has shown in a later paper, this retardation of growth may be due to overfeeding with thyroid tissue.

A. STANDARD U. S. P. PRODUCTS[24]

The cretins were produced experimentally according to the method described above. Eight cretins from four litters were fed with the desiccated thyroid. As soon as the symptoms of cretinism appeared, which was usually at the beginning of the third or fourth week, thyroid feeding was started. The thyroid was weighed out in small-size

20. Koch, F. C.: On the Nature of the Iodin-Containing Complex in Thyreoglobulin, Jour. Biol. Chem., 1913, xiv, 101.

21. Kendall, E. C.: (a) Specific Physiological Activity of Certain Constituents of the Thyroid Gland, Proc. Soc. Exper. Biol. and Med., 1912, x; (b) A Method for the Decomposition of Proteins of the Thyroid, with a Description of Certain Constituents, Jour. Biol. Chem., 1915, xx, 501.

22. Schaefer, E. A.: The Effects upon Growth and Metabolism of the Addition of Small Amounts of Ovarian Tissue, Pituitary and Thyroid to the Normal Dietary of White Rats, Quart. Jour. Exper. Physiol., 1912, v, 3.

23. Gudernatsch, J. F.: Feeding Experiments on Tadpoles, Am. Jour. Physiol., 1914, xxxvi, 370; Am. Jour. Anat., 1914, xv, 431.

24. Armour's Standard U. S. P. Desiccated Sheep Thyroid was used. This preparation is fairly uniform in activity and contains approximately 0.2 per cent. iodin in organic combination.

gelatin capsules. Such capsules can be fed without much difficulty. The initial dose was 0.015 gm. given daily. Cretin rabbits are very susceptible to thyroid feeding. Toxic symptoms develop with much smaller doses than with the normal rabbit of the same size. This is evidently due to the absence of the thyroid gland, which normally has been shown by Jones and Tatum[4] to take the organic iodin out of the blood very quickly. The animals were weighed twice a week and if there was any evidence of toxicity as shown by the growth curve, and other symptoms, then the dose was reduced. This dose was gradually increased. By the twentieth week the amount given was on the average 0.075 gm. Great care had to be taken to prevent toxicity because

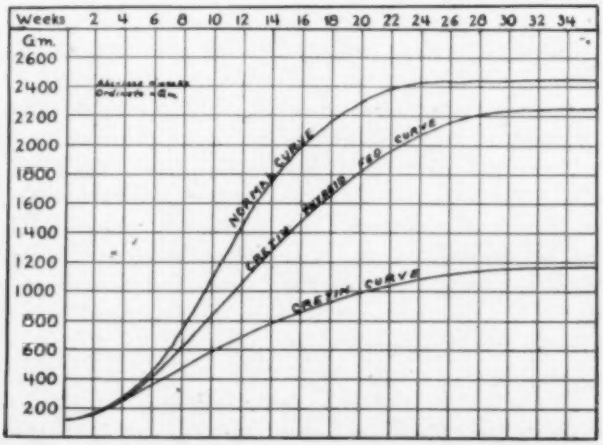

Fig. 7.—The growth curves of four normal controls, five cretins, and of eight cretins which were fed standard U. S. P. thyroid preparations.

usually the animal lost so much weight that it took several weeks merely to recover his original weight.

Figure 7 represents the average growth of seventeen rabbits. The upper curve represents the average growth of the four normal controls. In this connection it may be said that the variation in growth is negligible, making it unnecessary to plot separate curves. The growth gradually increases up to the twelfth week, after which there is a gradual decrease. This decrease is most marked from the twentieth to the twenty-fourth week. The average weight of four normal rabbits is 2,475 gm. The lower curve represents the average growth of the cretins of the four litters which had not been fed thyroid. The variation in weight in the four cretins was negligible. As can be seen in Figure 7, the growth curve increases up to the fifth week, after which

there is a gradual retardation of growth. This slight difference in weight from the normal from the second to the fifth week is evidently due to the operation. The maximum growth is reached at the thirtieth week (7½ months). The maximum average weight of the cretins was 1,175 gm. The maximum growth in the normal rabbit is reached about six weeks before that of the cretin rabbits. This was evident in all cretins under observation.

The second curve represents the growth of the eight cretin rabbits that had been fed thyroid. The curve is identical with the normal cretins up to the fifth week, after which there is a gradual increase, but this increase does not approach the normal curve. The maximum growth is reached about the thirty-second week (8 months) when the

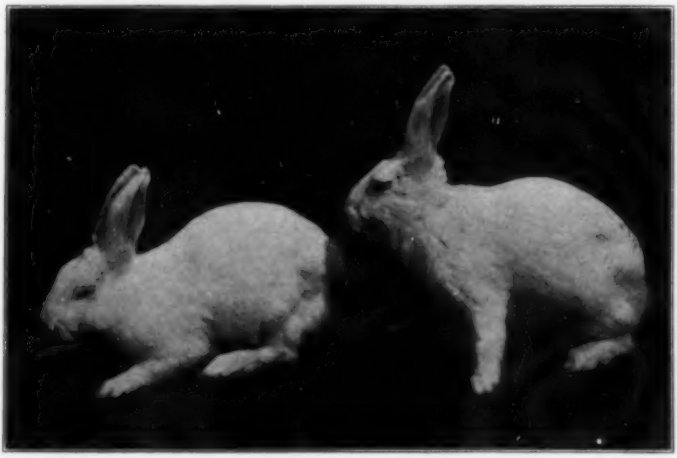

Fig. 8.—Normal control (right) and cretin from same litter fed standard thyroid preparation. Age, 20 weeks. Weight of control, 2,210 gm.; of fed cretin, 1,860 gm.

weight is 2,250 gm. *Growth continues about eight weeks after the maximum growth of the normal rabbit has been reached.* In no case was it possible to reach the growth of normal rabbits, as some investigators claim to have done. This was probably due to the fact that feeding experiments are difficult to control because of the lessened resistance to the thyroid feeding.

The symptoms of cretinism gradually disappeared. The hair became smooth and attained the moist, oily appearance of the normal rabbit. The scaly condition of the skin about the ears and legs disappeared. The pot belly, so characteristic of cretins about the eighth week, gradually disappeared. The animals became more active and

the weakness of the muscles, bones and joints was not noticeable after six weeks' feeding. Deformities of the bones never appeared in any of the thyroid-fed rabbits. This is evident from a comparison of the two rabbits in Figure 8. It represents the typical results of thyroid feeding. The age of the animals in the litter is 20 weeks. The normal rabbit on the right weighs 2,210 grams, while the left thyroid-fed rabbit weighs 1,860 gm. A comparison with the cretin rabbits which were not fed thyroid will show the absence of symptoms in the thyroid-fed rabbit. In all the cretins and thyroid-fed rabbits under consideration, no thyroid tissue was evident at necropsy.

Fig. 9.—Two thyroid-fed cretins. Feeding discontinued for five months.

When the thyroid feeding in the cretins is discontinued for some time, certain symptoms of thyroid deficiency soon become evident. This is evident from Figure 9. Thyroid feeding had been discontinued in the two rabbits for five months. They appeared perfectly normal when the thyroid feeding was stopped. The hair soon became dry, coarse, and could easily be pulled out in bunches. The skin became dry, scaly and gradually became eczematous. This was most evident on forehead, ears, legs and abdomen. The weakness of bones, muscles and joints also reappeared. The animals once more acquired the typical symptoms of cretinism, and except for their size, which was induced by thyroid feeding, could not be differential from cretins which served as controls.

B. FEEDING OF THYROID METAPROTEIN[25]

The cretins were produced as in the previous experiments. The results are based on seven rabbits in two litters having two controls and five cretins. It was found that this preparation was considerably more toxic than the standard U. S. P. thyroid, and it was necessary to reduce the dose to 0.005 gm., given daily to prevent toxicity. This dose was gradually increased to 0.05 gm. The cretins continued to grow till the end of the thirty-second week (eight months), when they attained the maximum weight of 1,950 gm. This period is a somewhat longer growth period than in the normal control.

Figure 10 represents the growth curves of the normal, normal cretins, and cretins which were fed the metaprotein compound. The

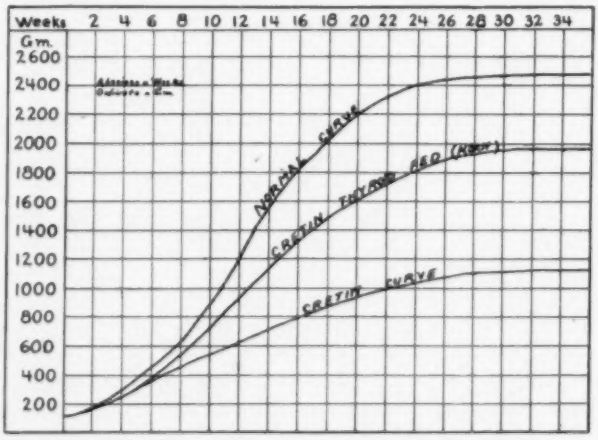

Fig. 10.—The growth curves of two normal controls, two cretins, and of three cretins which were fed Koch's metaprotein thyroid product.

normal and cretin growth curves are very similar to those of Figure 7. The middle curve represents the average growth of the three cretins fed with the thyroid metaprotein. There is a gra ual increase of the growth as early as a week following the use of the preparation. After the sixteenth week there is a gradual decrease of the growth. Comparing Figures 7 and 10, it is seen that the increase of growth is not as marked with feeding the metaprotein compound as with the standard

25. This thyroid split product was prepared by Dr. Koch in the biochemical laboratory. It is a metaprotein compound prepared from sheep thyroids and contains approximately 1.5 per cent. of iodin in organic combination. As tested by the acetonitril test, Koch found it considerably more active than standard thyroid preparations.

U. S. P. preparations. This may be explained partly by the increased difficulty of regulating the dosage because of the greater toxicity of the metaprotein over that of the standard U. S. P. product. When referring to toxicity, I have reference to the symptoms of overfeeding or "hyperthyroidism," namely, diarrhea, tachycardia, tremor and nervousness.

C. KENDALL'S THYROID EXTRACT B[26]

The animals used for these experiments were taken from two litters having three normal controls and five cretins. Three of these cretins were fed "Extract B." It was found that this preparation was non-toxic. There was no improvement either in the growth curve or in

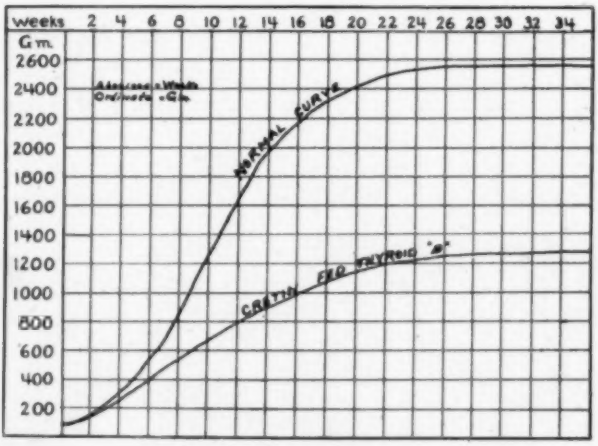

Fig. 11.—The growth curves of three normal controls and of five cretins, three of which were fed Kendall's Extract B.

any of the other symptoms of cretinism. Increasing doses were given up to 0.5 gm., with negative results.

In Figure 11, we have the growth curves of normal control and cretin rabbits which have been fed Extract B. This latter curve is identical with that of the normal cretin. These curves are very similar to those of Figure 1. As far as growth is concerned, these results agree with those of Kendall. As pointed out before, he claims its chief effect in man to be on the hair and skin. The weakness of muscles, bones and joints, and the mental activity are greatly improved,

26. Both the chemistry and its physiologic properties are described in the recent paper by Kendall.

according to Kendall. On the other hand, my results were entirely negative. This can be readily seen by comparing A and B of Figure 12. They represent two cretins and a normal control taken at the age of 7 and 20 weeks, respectively. A was taken at the age of 7 weeks, just before feeding was started. The weight of the control was 820 gm. and that of the cretins, 480 and 360 gm., respectively. B represents the same litter after twelve weeks (three months) of feeding with the thyroid Extract B. The weight of the control was 2,375 gm. and of the cretins 1,200 gm. and 1,250 gm., respectively. The hair, skin and pot belly show no improvement. The weakness of muscles, bones and joints is especially marked.

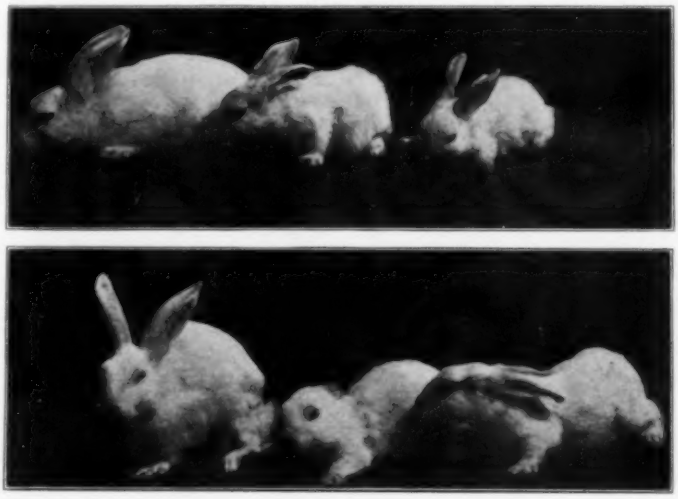

Fig. 12.—*A* (above) age 7 weeks. Normal control weighing 820 gm. and two cretins weighing 360 and 480 gm., respectively. *B* (below) same litter as *A* after feeding Kendall's Extract B. Age, 20 weeks. Normal weighs 2,375 gm. and cretins 1,200 and 1,250 gm., respectively.

IV. COMMENTS ON THE RESULTS

The feeding experiments show that experimental cretinism can be very effectively controlled by the use of standard thyroid preparations. It is evident from the experiments, that a very small dose given daily is sufficient to supply the physiological needs. This amount ranges from 0.015 gm. given daily at the beginning of the experiment, to 0.075 gm. at the age of 20 weeks. Toxicity from thyroid feeding is more readily induced in cretins than in normal rabbits of the same age.

Since it is known that the thyroid gland has a great chemical affinity for iodin and iodin compounds, there would probably seldom be an excessive amount of the active principle of the thyroid in the blood of normal rabbits. This probably accounts for the greater toxicity in thyroidectomized rabbits.

Cleavage products prepared from desiccated or fresh thyroids are still active if this hydrolysis does not go too far. Koch's metaprotein compound containing 1.5 per cent. iodin in organic combination is more active than standard U. S. P. desiccated thyroid containing about 0.2 per cent. iodin. Doses one-third the weight of the standard product are sufficient for physiological needs. That the activity of thyroid preparations depends, within certain limits, on the amount of iodin in organic combination, has been shown repeatedly.

Kendall's Extract B, although containing 40 per cent. of the total iodin, has no effect on the growth curve or on the other symptoms of cretinism; namely, that of hair, skin and weakness of muscles, bones, and joints. This preparation was found to be nontoxic to cretins and normal controls. Presumably, hydrolysis has gone too far, and the iodin is not in its normal organic combination.

Transfusion of normal blood serum has no effect on the growth and the other symptoms of cretinism. The animals remained identical with the cretins which were not transfused. No toxicity resulted from the injections. The negative results, however, do not disprove the theory of an active principle of the thyroid in normal blood. It was impossible to make more complete or more frequent transfusions. Furthermore, it is known that the equivalent of only a few milligrams of iodin in organic thyroid combination given daily is sufficient for normal physiological conditions. These results also agree with those of other investigators who have applied Hunt's acetonitril test to normal blood with negative results.

Transfusion of "hyperthyroid" blood serum increased growth considerably. The other symptoms, as that of the hair, skin, pot belly, weakness of muscles, bones and joints, also showed marked improvement. The increase in growth is, however, not as marked as with the feeding experiments. This growth curve never reached more than two-thirds that of the normal curve. The fact that we get marked improvement in cretins, however, is direct evidence of the presence of an active principle of the thyroid in the blood of "hyperthyroid"-fed rabbits. It must be granted, however, that these active principles of the thyroid probably represent the product absorbed from the gastro-intestinal tract and not that of an internal secretion of the animal's own thyroid gland.

V. SUMMARY

1. Transfusion of normal blood serum into cretins has no effect on the condition of cretinism.

2. Transfusion of "hyperthyroid" (thyroid-fed animal's) blood serum into cretins is effective in increasing the growth and in controlling the other symptoms of cretinism. The improvement, however, is not as marked as with the thyroid feeding.

3. Standard thyroid preparations (containing 0.2 per cent. iodin in organic combination), when given in carefully controlled nontoxic doses, will increase the growth of cretin rabbits and prevent the development of, or counteract, the other symptoms of cretinism. But thyroid feeding fails to carry an absolute cretin to full normal stature. Discontinuing the thyroid feeding leads to a return of some of the cretin symptoms.

4. The thyroid metaprotein of Koch is somewhat more active than standard thyroid preparations, but also more toxic.

5. Kendall's thyroid Extract B has no effect on any of the symptoms of cretinism. It is nontoxic, at least in ordinary doses.

6. Cretins are more susceptible than the normal animals to the toxic action of thyroid (thyroid feeding).

7. Cretin rabbits, despite their retarded rate of growth, continue to grow for a considerably longer time (four to six weeks) than the controls of the same litter.

I wish to express my great appreciation to Dr. Carlson for valuable suggestions and criticisms; also to Drs. Koch and Kendall who kindly gave me samples of their thyroid cleavage products for use in my experiments.

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AGGLUTINATION IN PERTUSSIS

ITS CHARACTERISTICS AND ITS COMPARATIVE VALUE IN CLINICAL DIAGNOSIS, AND IN DETERMINATION OF GENUS AND SPECIES *

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ASSISTED BY
EDWARD WORTH
NEW YORK

INTRODUCTION

The work herewith reported is part of a series of studies on pertussis begun by the Bureau of Laboratories of the New York City Health Department in 1914 under direction of Dr. A. W. Williams.[1] The subject of agglutination was taken up chiefly because of the different reports given by various authors as to the value of agglutination and of complement fixation in the early clinical diagnosis of pertussis, and because of the comparatively few definite reports made on the value of former test.

CRITICAL STATEMENT OF PREVIOUS FACTS

All authors (at least those who give some details about their work) are agreed that agglutinins for *B. pertussis* may be produced in most test animals but statements are vague as to the time and amounts of production and the condition under which they are produced.

The general statement is usually made that after several inoculations made preferably into the peritoneal cavity, the serum of the animal showed agglutinins. The reports on the strength of the serum, if stated at all, is varied. Thus Bordet and Gengou[2] obtained in rabbits after four inoculations intraperitoneally a serum which the authors say was not as strong as that of the horse which was inoculated many more times subcutaneously and intravenously. Arnheim[3] obtained a serum in rabbits of 1 to 5,000 strength and in the horse 1 to 10,000, but he gave no details of methods and times of inoculation. Wollstein[4] by

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1. Williams, Anna Wessels: The Etiology of Pertussis, Arch. Pediat., 1914, xxxi, No. 8.

2. Bordet, J., and Gengou: Note Complementaire sur le microbe de la coqueluche, Ann. d. l'Inst. Pasteur, 1907, xxi, 720.

3. Arnheim, Georg: Ueber den gegenwartigen stand der Keuchhustenfrage, Berl. med. Wchnschr., 1908, xlv-II, 1453.

4. Wollstein, Martha: The Bordet-Gengou Bacillus of Pertussis, Jour. Exper. Med., 1909, xi, 41.

intraperitoneal inoculations obtained a serum in the rabbit up to 1 to 800. Intravenous inoculations were not successful. No details of amounts or time of inoculation are given. Bächer and Menschikoff,[5] Shiga, Imai, Euguchi[6] found agglutination tests in pertussis of no value, as their bacillary emulsions were self-agglutinating.

Wollstein,[4] Frankel,[7] Seiffert,[8] Arnheim,[3] found agglutination tests of some value in clinical diagnosis of pertussis. Renaux[9] did not think it worth while to try.

Bordet[10] finds it of value only under certain conditions, namely, the organism must be freshly isolated from human sputum and grown on rich blood medium. The work of Bordet on agglutination will be dwelt on more in detail later.

ORIGINAL WORK

Our studies may be divided into the following:

1. The determination of the best conditions under which agglutinating serum may be obtained in test animals.

2. The comparative value of the test in demonstrating specificity of strains isolated by us from human beings suffering from whooping cough.

3. To add further evidence as to the comparative value of complement fixation and agglutination in the clinical diagnosis of pertussis.

1. *The Production of Agglutinins.*—Rabbits, horses, goats and sheep were used by us in producing agglutinins. Rabbits and horses responded more readily than the goats and sheep, so most of the tests have been made with serums from the former animals. Practically all of the rabbits inoculated (over 95 per cent.) which have remained in good health have produced abundant agglutinins for *B. pertussis*, though not all equally abundant.

Antigens Used.—We have worked with twenty-seven strains of culturally and morphologically typical Bordet-Gengou bacilli (*B. pertussis*), with nine strains of *B. influenzae*, with four strains of *B. bronchisepticus*, and with four

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7. Fraenkel, C.: Untersuchungen zur Entstehung des Keuchhusten, München. med. Wchnschr., 1908, lv, 1683.

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9. Renaux, E.: Le microbe de Bordet et Gengou agent etiologique de la coqueluche, Centralbl. f. Bact., etc., 1914, 1 Abt. Orig., lxxv, No. 3, 197.

10. Bordet, J.: Note Complementaire sur le microbe de la coqueluche et sa variabilite au point de vue du serodiagnostic et de la toxicite, Centralbl. f. Bact., 1912, Abt. 1, Orig., lxvi, Parts 2-4, p. 276.

strains of bacilli morphologically similar to *B. pertussis*, but with cultural differences. These strains we call pertussis-like bacilli. Living emulsions were used for the regular inoculations. In the rabbit, antigen *extracts* similar to those used in the complement fixation test were also tested, as were regular vaccines and sensitized vaccines, for their power to produce agglutinins.

Living cultures of B. Pertussis for inoculations were prepared by *scraping* off a forty-eight-hour culture grown on tube slant on Bordet-Gengou medium in recently isolated cultures, and on coagulated horse blood glycerin veal agar in older strains, and emulsifying in normal salt solution. The dose was graduated according to age of the culture from isolation and to the kind of culture medium used.

The highest virulence for guinea-pigs has been one-half slant of a profuse culture inoculated intraperitoneally. This initial virulence on the blood-veal-agar medium became less after the culture had been grown in successive transplants on this medium for a year. Then one or two slants on coagulated horse blood agar were required to kill a guinea-pig of about 200 strains inoculated intraperitoneally.

The living cultures of influenza bacilli were grown for forty-eight hours on coagulated horse blood agar and then scraped off and emulsified in normal salt solution.

The initial dose of *B. pertussis* and of *B. influenzae* inoculated intraperitoneally into rabbits was from one-eighth to one-half slant. The last dose was five to six cultures on 100 c.c. Blake bottles.

The *pertussis-like* strains were all on plain veal agar, on which medium they grow abundantly. The emulsions were made in the same way as the others. These strains were very virulent for rabbits, one especially, No. 31, which had to be given at first in doses of $\frac{1}{100}$ to $\frac{1}{50}$ of a slant. The last dose was four slants.

The *antigen extracts* were prepared in the same manner as were those for complement fixation tests: namely, a forty-eight-hour growth of *B. pertussis* on coagulated horse-blood-veal-agar medium was scraped off in distilled water, shaken for two successive days, brought up in the water bath to 56 C. and left in the incubator at the same temperature over night; the next day the emulsion was centrifuged and the supernatant fluid rendered isotonic by adding 9 per cent. of salt and was injected into the animals.

The dosage of this antigen was ¼ c.c. intravenously, ½ c.c. intraperitoneally, or 1 c.c. subcutaneously. The dose was gradually increased up to 10 c.c. The toxicity of this antigen was tested and it was found that 5 to 10 c.c. of it killed guinea-pigs 200 gm. in weight, inoculated intraperitoneally, in twenty-four hours; 3 c.c. had no effect.

The *vaccines* were made by scraping a forty-eight-hour growth of *B. pertussis* on coagulated horse blood veal agar, emulsifying in physiologic salt solution, shaking the emulsion for two successive days and then heating it at 56 C. for one and one-half hours. The initial dosage of the vaccine, which was usually 15 billions per cubic centimeter strong, was ¼ c.c. intravenously, ½ c.c. intraperitoneally, or 1 c.c. subcutaneously. Its toxicity was such that 10 c.c. of a 15 billion strong vaccine killed guinea-pigs of about 200 gm. weight, inoculated intraperitoneally, in twenty-four hours; 3 to 5 c.c. had no effect.

The *sensitized vaccines* were prepared the same way as the killed vaccines, except that they were shaken only one day, and instead of being heated were agglutinated by 1:100 immune pertussis serum for one hour in the incubator at 37 C. and were then placed in the ice-box over night. The next day the precipitated emulsion was shaken up (the supernatant fluid is quite clear) and washed two to three times in physiological salt solution. The dosage of this vaccine was the same as of the killed one, but it was usually 10 instead of 15 billions per c.c. strong. Its lethal dosage is still being tested in order to gain more explicit data about the comparative virulence of living and sensitized pertussis

organisms. Thus far we have found that 1:40 immune rabbit pertussis serum added to 3 c.c. of a 10 billion per c.c. strong vaccine prevents the death of 200 gm. guinea-pigs, while the control mixtures of normal rabbit serum added to the same amount of emulsion killed the pigs by the next day. The emulsions of nonsensitized bacilli in 1 c.c. doses killed the pigs in twenty-four hours. The sensitized vaccine organisms remain alive in the ice-box for two to three weeks, when the growth gradually decreases; on the fifth week only a few colonies are obtained. The untreated organism lives in the ice-box about two months. More work is being done on the bacterial toxicity of these immune serums.

Technic of Agglutination Tests.—It is of vast importance to obtain homogeneous emulsions, especially for microscopic tests. The importance of the culture medium and the subsequent treatment of the cultures for agglutination tests has thus far never been dwelt on in detail by any writer on this subject, except perhaps Bordet[11] himself, and then only on some phases of it. *B. pertussis* when recently isolated grows only on Bordet-Gengou medium, but after five to six generations, it grows much more abundantly and characteristically on glycerin veal agar 0.4 acid to phenolphthalein, to which horse's blood (1 to 75 to 1 to 100) has been added while the agar is very hot (90). The influenza bacillus also grows excellently on this medium, with the exception that the reaction should be neutral to phenolphthalein. Though the pertussis organism grows equally well on the neutral medium, it forms an adherent growth which cannot be separated from the medium and the acid reaction seems to prevent this occurrence. The cultures for the tests are taken from a forty-eight-hour growth on this acid medium and allowed to grow for twenty-four hours on 1 to 100 coagulated horse blood veal agar *neutral* in reaction. A twenty-four-growth usually is not adherent on neutral medium in the first generation from the acid medium. The glycerin is omitted for the last growth, as it seems to interfere with the reaction. Recently isolated strains, of course, must be grown on Bordet-Gengou medium. The growth is scraped off, not washed off, in distilled water and shaken in an electrical shaker for three to four hours. A standard emulsion is kept in the ice-box, so that the emulsions are always uniform. The serums are diluted with 0.85 per cent. salt in 1 to 10, 25, 50, 100, 200, 400 parts, etc. Each test tube contains ¼ c.c. of the diluted serum and ¾ c.c. of the emulsion, the final dilution being 1:4. The tests are macroscopic with the animal serum and microscopic with patient's serums unless there is enough serum for the macroscopic test. The test tubes and hanging drops are put in the incubator at 37 C. for one to two hours and readings made; the macroscopic tests are left over night in the ice-box. Complete agglutination is designated by ++; incomplete by +; slight by ±.

With this technic we have not as yet encountered a self-agglutinating strain on coagulated horse-blood-veal-agar medium and only in the very beginning a few recently isolated strains on Bordet-Gengou medium were slightly self-agglutinating (perhaps they were not shaken long enough). The glassware must be absolutely clean and antiseptics such as mercury chlorid avoided. For the examination of patients' serums, one or more strains of *B. pertussis* of known agglutinability and one influenza strain are used. One recently isolated strain is grown on rich Bordet-Gengou medium (according to the suggestion from Bordet); the other on our standard medium (coagulated horse

11. Bordet, J., and Sleeswyk: Serodiagnostic et variabilite des microbes suivant le milieu de culture, Ann. d. l'Inst. Pasteur, 1910, xxiv, 476.

blood veal agar). The last medium as a rule gives the most satisfactory results, especially for hanging drops, as it invariably forms a homogeneous mass of entirely separated organisms, and when a reaction occurs it is quite unmistakable.

First Appearance of Agglutinins.—Agglutinins, as a rule, did not begin to appear in the rabbit until after the sixth or seventh inoculation; at least in no higher strength than 1:40 or 1:100. After that, if the animal kept well, the agglutinins continued to rise with each inoculation. Most rabbits were sacrificed when they showed from 2,400 to 5,000 titer, which happened usually after the tenth to the twelfth inoculation. Some of them might possibly have shown a higher titer if further inoculated.

Normal rabbits show agglutination in dilution not higher than 1:25; sheep and goats 1:40 to 1:100; horses up to 100. The sheep and goats seemed to respond more slowly to the inoculations than the rabbits and horses, so they were dropped. Two of the three horses—one by intravenous, the other by subcutaneous inoculation—showed agglutinins up to 2,000 after the third and fourth inoculation. Two horses are still in the process of immunization.

The injection of living cultures intraperitoneally seems to be most conducive to the development of agglutinins in the rabbit, though now and then there is a rabbit found which shows fairly good agglutinations after four to six inoculations. With killed vaccines, sensitized vaccines and antigens, the titer has never become as high as with living cultures.

Some of the rabbits inoculated with killed vaccines and more of those inoculated with sensitized vaccines and antigens (eight to nine inoculations each) did not develop agglutinins to any extent, though they were markedly immune. Thus, five of such rabbits were injected with four to five surface growths of 100 c.c. bottles without ill effects, while the control rabbits died from the same dose or less (three bottles) the same day or next morning from acute toxemia. Two rabbits, more responsive to the vaccines, developed after a few inoculations a comparatively high agglutination titer—up to 1,000. The low titer rabbits were inoculated later with living cultures, and after a few inoculations showed a high agglutinating power. The influenza rabbits did not form agglutinins as readily as did the pertussis rabbits, and the titer never reached higher than 1 to 1,600. The accompanying table shows the comparative rise of agglutinins and of complement fixing bodies in rabbits inoculated with *B. pertussis, B. influenza* and pertussis-like bacilli.

2. *Specificity of Strains from Whooping Cough Cases.*—From Table 3, which shows the agglutinative reaction of pertussis serums

TABLE 1.—Comparative Rise of Agglutinins and Complement Fixation Antibodies, with—Intraperitoneal—

Initial Wt. Gm.	Last Wt. Gm.	Initial Dose	Last Dose	Strain	After 3d Inoculation		After 4th Inoculation		After 5th Inoculation		After 6th Inoculation		After 7th Inoculation	
					Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.
PERTUSSIS RABBITS														
1,920	1,840	⅛ slant	8 slants.....	P. D.	†	.001	1:40	.0003	Rabbit sacrificed after four inoculations					
2,400	2,670	¼ slant	Surface grown of 3-100 c.c. bottles	55	0	0	0	0	0	.005	†	.003	1,200	.002
1,420	1,780	⅛ slant	Surface grown of 4-100 c.c. bottles	93	0	.005	0	.004	0	.003	0	.001	†	.0009
1,380	1,750	¼ slant	Surface grown of 4-100 c.c. 3 bottles	110	0	.004	0	0	0	.005	†	.0004	400	.0007
2,040	1,880	1 slant on plain veal agar	Surface grown of 4-100 c.c. 12 bottles	P. D.	0	0	0	0	40	0	1,200	.001	2,000	.004
1,345	1,565	¼ slant	Surface grown of 4-100 c.c. 4 bottles	154	0	0	0	0	0	0	0	0	0	0
1,870	2,300	½ slant	Surface grown of 4-100 c.c. 9 bottles	55 M93	0	0	0	0	200	0	1,000	.001	2,000	.005
1,700	2,130	Sensitized vaccine ⅛ c.c. ¶	Living culture surface grown 6-100 c.c. bottles	154 155 163	0	0	0	0	40	0	†	.02	200	.002
1,960	2,110	Extract "antigen" 1 c.c.	Living culture surface grown 4-100 c.c. bottles	154 155 163	0	0	0	0	0	0	0	0	0	0
2,670	2,730	Killed vaccine ⅛ c.c.	8 c.c.....	154 155 163	0	.003	0	†	400	.002	1,600	.0005		
INFLUENZA RABBITS														
1,910	2,250	¼ slant	Surface grown of 8-100 c.c. bottles	37	0	0	0	0	0	0	0	0	0	0
1,430	1,660	½ slant	Surface grown of 5-100 c.c. bottles	BI2	0	0	0	0	0	0	0	.003	0	.003
1,810	2,060	¼ slant	Surface grown of 8-100 c.c. bottles	35	0	0	0	0	0	.004	0	.003	0	.004
2,220	2,300	⅛ slant	Surface grown of 11-100 c.c. bottles	747	0	0	0	0	0	0	0	0	0	.003
RABBITS INOCULATED WITH PERTUSSIS-LIKE BACILLUS														
1,905	1,710	1/50 slant	3½ slants.....	31	0	.004	0	.003	0	.005	0	.009	†	.009
1,960	2,365	⅛ slant	6 slants.....	C.	0	0	0	.005	0	0	0	.006	0	.007
1,565	1,620	⅛ slant	1 slant.....	1	†	†	1,200	.0006	Sacrificed					

* Injections with living cultures were given once a week; vaccines and antigens were given twice a week till the sixth inoculation, then once a week.
† Not tested.
‡ After injection with living cultures intraperitoneally.

—Successive Inoculations of B. Pertussis, B. Influenzae and Pertussis-Like Bacillus by —Injections

After 8th Inoculation		After 9th Inoculation		After 10th Inoculation		After 11th Inoculation		After 12th Inoculation		After 13th Inoculation		After 14th Inoculation		After 15th Inoculation	
Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.	Agglut.	C. F.
PERTUSSIS RABBITS															
†	.002	1,000	.001	2,400 no more dilutions tried	.001	5,200	Rabbit sacrificed								
2,400	.001	5,200	Rabbit sacrificed												
1,200	.0004	1,000	Rabbit sacrificed												
2,400	.0005	2,000	.002	2,000	.0004	2,400	.008	2,400	.0008	3,200	.002				
0	.001	†	.001	800	.004	1,200	0	1,000	0	800	0				
†	.0008	2,400	.001	2,400‡	.0008	4,000	.0008	10,000	.0008	Sacrificed					
†	.008	400	.001	2,000‡	.002	2,400	.002	2,400	.002	†	.002	4,000	.001		
0	0	40	.008	100	0	†	.006	200	0	1,200	.008	2,400	†	6,000	.001
INFLUENZA RABBITS															
0	0	0	.006	0	0	200	.001	40	.008	400	Test unsatisfactory .0008				
0	.001	40	.0007	0	.008	200	.001	200	.002	200		40	.0005		
0	.002	0	.0000	200	.0008	†	†	0	.0009						
200	.0008	200	0	†	†	40	0	200	.006	200	.006	40	†	200§	.005
RABBITS INOCULATED WITH PERTUSSIS-LIKE BACILLUS															
1,200	.009	2,400	.001												
0	.005	0	.008	0	.006	0	.008								

§ After the seventeenth inoculation this rabbit reacted up to 1:1600; after the eighteenth inoculation it dropped down to 1:400; at the end of the twenty-first inoculation the reaction was only 1:200. Complement fixation varied from 0.0008 to 0.0001.

¶ Subcutaneously.

TABLE 2.—Agglutination Tests of Pertussis Serum with Homologous and Heterogeneous Strains—B. Influenza, Pertussis-Like Bacillus and B. Bronchisepticus

Pertussis Strain	Titer of Agglutination in Incubator at 37 C. for 1½ Hours	40	400	2,000	2,400	2,800	3,200	4,000	5,000	6,000	7,000	8,000	10,000	Control
55	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
M.	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
93	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
96	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
P. D.	8,000	++	++	++	++	++	++	++	++	++	++	++	++	—
162	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
155	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
154	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
109	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
188 R.	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
100	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
98	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
110	2,000	++	++	++	++	++	++	++	++	++	++	++	++	—
114	2,000	++	++	++	++	++	++	++	++	++	++	++	+	—
Influenza strain*	Tried in dilution 1:40, 100, 200, 400, etc.	....	....	....	....	....	....	....	....	....	....	....	....	..
747	—	....	....	....	....	....	....	....	....	....	....	....	....	..
37	—	....	....	....	....	....	....	....	....	....	....	....	....	..
33	—	....	....	....	....	....	....	....	....	....	....	....	....	..
35	—	....	....	....	....	....	....	....	....	....	....	....	....	..
Pertussis-like strains*		....	....	....	....	....	....	....	....	....	....	....	....	
31	1:40, 100, 200 + — —	+	—	—	....	....	....	....	....	....	....	....	....	..
C.	1:40, 100, 200 + — —	+	—	—	....	....	....	....	....	....	....	....	....	..
10	— — —	—	—	—	....	....	....	....	....	....	....	....	....	..
1	+ — —	+	—	—	....	....	....	....	....	....	....	....	....	..
Broncho strains*		....	....	....	....	....	....	....	....	....	....	....	....	..
155	40, 100, 200 ± — —	±	—	—	....	....	....	....	....	....	....	....	....	—
156	± — —	—	—	—	....	....	....	....	....	....	....	....	....	—
97	++ ± ± Control ±	±	±	±	....	....	....	....	....	....	....	....	....	±

* Pertussis serum.

with heterogeneous strains and attempt at cross agglutination with *B. influenza,* and pertussis-like bacilli, it would seem that not only do the pertussis strains show no relationship to the pertussis-like strains or to the influenza strains, but that all strains of typical pertussis bacilli belong to one group. This is in marked contrast to the reaction of influenza serums which agglutinate most heterogeneous strains in very low dilutions or not at all. The pertussis-like organisms also show a variation in strains. Thus of the four strains tried, No. 31 and No. 1 seem to be identical. The others seem to be quite distinct strains. In

TABLE 3.—PATIENTS' PERTUSSIS SERA

Weeks Whoop	Agglutination Reactions					Weeks Whoop	Complement Fixation Tests				
	No. of Cases	—	1:40	1:100	1:200		No. of Cases	—	+	++	+++
1	13	5	4	..	4	1	6	4	0	1	1
2	10	0	6	3	1	2	4	3	0	0	1
3	9	1	0	6	2	3	5	0	2	2	1
4	13	2	4	6	1	4	2	0	1	0	1
5	2	1	0	0	1	5	4	1	0	1	2
6	4	0	4	0	0	7	2	2	0	0	0
14	1	0	0	1	0	17	1	0	0	0	1
20	1	1	0	0	0	Convalescent 8	1	..	..	..	1
Convalescent 1	2	1	0	1	..	Convalescent 5 mos.	1	..	..	..	1
Convalescent 8	1	1	..	..	..	.....	..	..	..	..	..
Convalescent 5 yrs.	1	1	..	..	..	.....	..	..	..	..	..
Exposed to W. C. 2 weeks	2	..	2	..	..	.....	..	..	..	..	..
Control sera, adults	58	29	19	10	..	.....	51	49	2	..	..
Control sera, children (scarlet fever)	10	6	4	..	..	.....	..	..	..	..	..

no instance is there cross agglutination of *B. pertussis* serum with *B. influenza,* even in 1:40. The serum of the pertussis-like bacilli and the influenza serums sometimes agglutinate *B. pertussis* in 1:40, but never higher. Of the four strains of *B. bronchisepticus* used, none showed any relationship to pertussis agglutinins. The variability of hemoglobinophilic strains has been shown by previous work.[12] *Absorp-*

12. Povitzky, Olga R.: Tests of Hemoglobinophilic Bacilli from Conjunctivitis in Regard to Their Virulence and Their Agglutinating Properties, Collected Studies, 1913-1914.

tion tests verified the supposition gained from the straight agglutination tests, that all pertussis strains belong to one species as far as agglutination reactions are concerned. In order to do these tests, the serum must be saturated by a very thick emulsion, as the least agglutinin not absorbed may be shown markedly by sensitive strains more agglutinable than others, and in this way, obscure the test. One rabbit was inoculated with three strains which we thought might be a little different from each other on account of their varied powers to agglutinate in weak serums, but by absorption tests they proved to have identical agglutinins for themselves as well as for all the other strains[10] tested.

Variations in Agglutinability of Pertussis Strains.—Though all pertussis strains are practically equally agglutinated by a strong serum, individual strains vary in regard to promptness of response, and with a weak serum heterogeneous strains may show marked difference in the quantity of serum required for a reaction. We have often seen the phenomenon observed by Bordet and others, of the agglutination of heterogeneous strains more strongly than the strain with which the antiserum was obtained. This characteristic of *B. pertussis* is brought out more clearly by weak serum. Thus, some of our serums from rabbits and goats, after a few inoculations, hardly agglutinated their homologous strains, while they agglutinated a heterogeneous strain up to 1 to 2,000. It is important, therefore, in testing patients serums to use a strain of known agglutinability (as in typhoid).

The difference in the agglutinability of different pertussis strains was well brought out in the instance of one of our rabbits inoculated with three strains, M., 55 and 93. After five inoculations, its serum agglutinated strains 93 and M. and the heterogeneous strain P. D. up to 200, but not at all Strain 55. After six inoculations, M., 93 and P. D. were agglutinated up to 2,000, and 55 only very slightly in the first few dilutions. After the ninth inoculation, 55 was agglutinated up to 200, while the other strains showed very strong agglutination up to 2,400. After the tenth inoculation, all four strains (M., 55, 93, P.D.) showed very strong agglutination up to 2,400 (no more dilutions were tried). After eleven inoculations, all strains were agglutinated up to 4,000 (no higher dilutions tried). After the twelfth inoculation, agglutinins for all three hemologous strains and twelve other strains tried were 1 to 10,000 in a trial serum. It would seem that only a very strong serum could overcome the sluggishness of Strain 55. Another strain of this kind is No. 163. Rabbits inoculated with 155, 154 and 163 showed very high agglutination with both Strains 154 and 155 before 163 was agglutinated.

Another interesting feature in the agglutinations of *B. pertussis* is that when a mixture of strains is used in making the emulsions for agglutination, the reaction occurs more promptly than when each strain is used alone. Why this occurs we do not yet know. Because of it, we are making comparative tests with a mixture of three or more pertussis strains in emulsion and a one-strain emulsion for testing patients' serums.

According to Bordet,[10] the age of a culture from date of isolation influences the agglutinating property of *B. pertussis*. He cites an experiment in which he tested a pertussis serum stored in the ice-box for four years with its homologous strain kept transplanted all these years on Bordet-Gengou medium. No agglutination occurred though this strain was highly agglutinated by the same serum four years ago. The natural conclusion at first was that the serum had lost its potency, but when it was tested with a freshly isolated strain it gave a strong agglutinating reaction. Bordet, therefore, advocates for testing patients' serums for agglutination to use freshly isolated cultures grown on rich blood medium.

In our experience we have found that while freshly isolated strains on Bordet-Gengou medium are quite responsive to a strong serum, the most readily agglutinable strains are a few that were isolated one to one and one-half years ago, and which grow best at present on our standard coagulated horse-blood-veal-agar medium. Of course we cannot predict how much modified these strains may be in four years.

Relationship of Culture Mediums to Agglutination.—Bordet[11] states that animals inoculated with *B. pertussis* grown on Bordet-Gengou medium develop agglutinins for *B. pertussis* grown only on the same medium, not for the organism grown on plain agar, and vice versa. He also states that by absorption tests he found that these two agglutinins were not the same.

In testing these experiments, we worked with three mediums: Bordet-Gengou, coagulated horse-blood-veal-agar, and plain veal-agar (only two of our strains grow well on plain agar thus far). When working with weak serums (serums which do not give a prompt reaction in the incubator at one and one-half hours, though they may agglutinate up to 2,400 the next morning) and freshly isolated strains, we were impressed by the truth of Bordet's statements. Thus Horse 1, injected three times with two strains grown on Bordet-Gengou medium, did not agglutinate the same strains grown on coagulated horse blood veal agar, but agglutinated these very strains up to 2,000 when they were grown on Bordet-Gengou medium; but the same serum agglutinated our older agglutinable strain grown on coagulated horse blood veal agar in just as high dilutions. On the other hand, a rabbit

inoculated with a well agglutinable strain grown on plain veal agar after six to seven inoculations, agglutinated up to 2,000 equally well the same strain grown on each of the three mediums. Absorption tests did not seem to bear out the statement that the agglutinins were different. From our experience it would seem that the culture medium influences an agglutinable strain in so far as it affects its growth and best development, not in its production of different kinds of agglutinins. The recent strains grow well only on Bordet-Gengou medium and are, therefore, more agglutinable on this medium than when grown on coagulated horse blood veal agar, on which they grow in the beginning very scantily. The sluggish strains which grow well on either medium seem to be influenced by the culture mediums when tested with weak serums. Thus, the serum of the same horse injected with cultures on Bordet-Gengou medium, which agglutinated an easily agglutinable old strain on coagulated horse blood veal agar, did not agglutinate a sluggish strain on the same medium (though it grows on it profusely) but agglutinated it when it was grown on Bordet-Gengou medium.

A strong serum, therefore, in our experience, agglutinated equally well, though may be not equally promptly, all strains, sensitive and sluggish, no matter on what medium grown.

Comparative Value of Complement Fixation and Agglutination Tests in the Differentiation of Strains.—Agglutinins as a rule (as mentioned before) did not begin to show in the rabbit in any marked degree till after the sixth or seventh inoculation, while complement fixation antibodies, as seen from the accompanying table, usually appeared after the fourth inoculation, but also showed a higher titer after the eighth to ninth inoculation. Though complement fixation and agglutination outside of the time limit ran quite parallel, namely, most of the animals which show high complement titer show also strong agglutinins, there were rabbits which showed high complement titer and no agglutination, and at times vice versa. Especially was this the case when the rabbit showed a very high complement titer after a few inoculations. Thus Rabbit 28 inoculated with *B. pertussis,* after the fourth inoculation showed a complement titer of 0.0003, and agglutination only 1 : 40. On the other hand, Rabbit 92 showed complement titer of 0.001 while it agglutinated up to 5,200. Many influenza rabbits showed a high complement titer and very little or no agglutination. On the whole, however, agglutinins in pertussis rabbits, though produced more slowly, were produced more regularly and more surely than were complement-fixing substances.

From our observations we conclude that the agglutination of *B. pertussis* by artificially produced immune serum gives a more clear-cut differentiation from pertussis-like bacilli and from hemoglobino-

philic bacilli than does complement fixation which shows a cross fixation in low dilutions with some of these latter organisms (Olmstead and Povitzky[13]).

3. *The Comparative Value of Agglutination and Complement Fixation in the Clinical Diagnosis of Pertussis.*—Serums from 59 cases of pertussis in various stages of the disease were tested for agglutinins. These results, also the results of control serums of adults suffering from different diseases, and of children with scarlet fever are given in Table 3.

We see by this table that we cannot depend on agglutination as a positive guide to diagnosis in dilutions up to 1 to 100. If, however, the reaction at 1 to 100 is very prompt (in an hour at 37 C.) and complete, it may be considered suspicious, since in none of the controls did the reaction recorded occur quickly. When a reaction occurs in dilutions of 1 to 200, it indicates a probable recent infection with *B. pertussis.*

Unfortunately, we did not always get enough serum from patients to test both agglutination and complement fixation, so our comparative table is not of the same cases, but the series of those tested for complement fixation is taken from those quoted in the paper of Olmstead and Luttinger[14] that did not receive vaccines. According to this tabulation, a positive specific complement fixation did not occur quite as frequently in the first week as did agglutination at 1 to 200. On the other hand, out of fifty-one control serums of adults, only two showed a positive reaction by complement fixation while about 33 per cent. showed a reaction up to 1 : 40 by agglutination tests and about 17 per cent. showed a reaction 1 : 100. Control children serums showed in 40 per cent. a reaction in 1 : 40.

Only three out of the fifty-nine pertussis serums showed agglutination with *B. influenzae,* two in 1 : 40 and one in 1 : 200 in the first week of the whoop.

The control serums showed agglutination with *B. influenzae* only in two cases in 1 : 40, while complement fixation showed fixation with influenza quite frequently.

SUMMARY

1. A strongly agglutinating pertussis serum was best obtained in the rabbit by ten to twelve intraperitoneal inoculations of living cultures given at seven-day intervals. Over 95 per cent. of rabbits, inoculated in this manner, produced agglutinins, though not in equal abun-

13. Olmstead, Miriam P., and Povitzky, Olga R.: The Complement Fixation of Bordet-Gengou Bacillus, Jour. Med. Research, 1915.

14. Olmstead, Miriam P., and Luttinger, Paul: Complement Fixation in Pertussis, The Archives Int. Med., 1915, xvi, 1.

dance. Agglutinins are also produced by vaccines, but not as abundantly as by living cultures. Those rabbits which received eight to nine inoculations of vaccine and did not show agglutinins to any large extent, were, nevertheless, markedly immune.

A high titer serum (from 1:4,000 to 1:10,000) always proved to be a quickly acting serum which reacted in from one to one and one-half hours in the incubator at 37 C., sometimes up to 1:8,000, with well agglutinable pertussis strains.

A low titer serum was usually slow in reaction and did not show agglutination till after remaining in the ice-box over night.

2. By agglutination tests, *B. pertussis* strains can be specifically identified from hemoglobinophilic bacilli, pertussis-like bacilli, and *B. bronchisepticus*. In no instance was there cross agglutination between these organisms—at least not higher than 1:40. For identification a strong serum of high titer should be used.

3. By these tests it would also seem that all pertussis strains isolated in our laboratory belong to one group. A strong serum agglutinated all our pertussis strains (twenty-seven in number) equally well though not equally promptly in the highest dilution. Absorption tests (tried in seven most variable strains), proved the supposition gained by the straight agglutination tests.

4. The variation of the agglutinability of pertussis strains with weaker serums depends very much on the age of the culture (dating from isolation) and culture medium. Freshly isolated strains are best agglutinable on Bordet-Gengou medium; older strains on our standard coagulated blood veal agar.

6. A strong serum agglutinates equally well, though not equally promptly, all strains, no matter on what medium grown and how sluggish the strain is.

6. Agglutination tests in clinical diagnosis of pertussis compares favorably with complement fixation only in the first week of the whoop. In the later stages of the disease complement fixation antibodies appear more frequently than agglutinins.

7. Thirty-three per cent. of adult nonpertussis serums show agglutination in up to 1:40; 17 per cent. in up to 100; 40 per cent. of children's nonpertussis serums (scarlet fever cases) showed a reaction in up to 1:40. A dilution of not less than 1:200 is necessary for a practically positive diagnosis of pertussis by agglutination test.

THE PERMANGANATE REDUCTION INDEX OF CEREBROSPINAL FLUID*

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Children frequently exhibit meningeal symptoms as a manifestation of diseases other than meningitis. The frequency of such symptoms seems to vary in inverse proportion to the age of the child. Thus, infants may show meningeal symptoms in association with almost any disease, while in older children, these symptoms are usually seen only in diseases involving the brain or meninges. Notwithstanding the value of a bacteriological examination, cell count and globulin estimation on spinal fluid, clear fluids are quite frequently obtained on which these examinations throw no light. The great variability in the cytology of the cerebrospinal fluid under the same conditions and the difficulty often encountered in demonstrating the presence of micro-organisms in a clear fluid, make every new aid to diagnosis welcome.

It is particularly in the cases with clear fluids that the permanganate reduction test of Mayerhofer is often of great assistance in diagnosis. The principle of the test, an old one used in water analysis, was adapted by Mayerhofer[1] for the examination of the cerebrospinal fluid. He found that because of their higher content of organic substances, pathological fluids would reduce a larger quantity of permanganate solution than normal fluids. The amount of decinormal permanganate solution, which, boiled for ten minutes in a strongly acid medium is reduced by 1 c.c. of cerebrospinal fluid, he called the Permanganate Reduction Index. This amount was usually below 2 in normal fluids, Mayerhofer placing the border line of normal and pathological fluids between 2 and 2.3. He applied the test particularly to fluids from patients with tuberculous meningitis, in which a high index was practically the rule.

We have determined this index on the cerebrospinal fluids obtained from patients presenting meningeal symptoms, admitted to the hospital for a number of different conditions. In some of the cases, several determinations were made. As a rule, we confined our

* Submitted for publication Oct. 20, 1915.

* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital.

1. Mayerhofer, E.: Zur Characteristik und Differential diagnose des Liquor Cerebrospinalis, Wien. klin. Wchnschr., 1910, xxiii, 651.

tests to fluids which were practically clear, also eliminating fluids which contained blood. A few examinations of turbid fluids are given simply to show the constancy of high index values in such fluids. We did not determine the index on various portions of the same fluid, as suggested by Mayerhofer, but used a sample of the entire fluid obtained at one lumbar puncture. The titration was done as soon as possible after withdrawal of the fluid, since, as the author of the test notes, changes occur in fluids on standing which give erroneous results.

While the determination of the Permanganate Reduction Index does not possess the conclusiveness of a specific test, indices usually fall into certain groups, eliminating or suggesting certain diseases. In our series of cases, a reduction index of over 2.5 in a clear fluid, almost invariably indicated the presence of an actual inflammatory process of the brain or meninges, usually a tuberculous meningitis. Other conditions which may, with clear fluid, give high indices, are encephalitis, serous meningitis, and poliomyelitis, practically the same conditions emphasized by Lucas[2] as giving similar cytological findings.

We examined 17 fluids (Table 1) from patients with tuberculous meningitis. Thirty single tests were done on these cases. Of these, 16 had an index above 3, 13 were between 2.5 and 3; only one was below 2.5. This case with an index of 2.3, which is the highest of the border line readings given by Mayerhofer, on a subsequent puncture gave an index of 3.1. While it is true that all of these cases could have been diagnosed without the test, in some cases, the early appearance of the high index is most striking.

A comparison of the cell counts with the reduction index of these fluids shows no definite relationship between the two. The cell counts varied from 30 to 500 per c.mm. While, in an individual case, the cell count may show corresponding fluctuations with the reduction index, a comparison of two separate cases shows no such similarity. Thus, in Case 1 with an index of 2.7, the cell count is 30, in Case 22 with an index of 2.5, the cell count is 406. Furthermore, the height of the reduction index seems to vary much more noticeably with the course of the disease than does the cell count. The counts were made on the fresh fluid, employing an ordinary white cell counter, using 10 per cent. acetic acid for the dilution. Our results lead us to confirm the inconstancy of cytological findings, as has been noted by Kafka[3] and others.

2. Lucas, W. P.: The Non-Specificity of the Cyto-Findings in the Spinal Fluid in Various Meningeal Conditions, Especially in Children, Am. Jour. Dis. Child., 1911, i, 230.

3. Kafka, V.: Ueber den heutigen stand der Liquordiagnostik, München. med. Wchnschr., 1915, lxii, 105.

TABLE 1.—FLUIDS FROM PATIENTS WITH TUBERCULOUS MENINGITIS

Case No.	Cell Count	Globulin	Index
1	30	+	2.7
2	125	+	3.6
	.....	.....	3.8
7	280	+	3.3
11	325	+	3.2
17	270	+	2.5
16	80	.....	.....
	150	+	3.0
	160	.....	.....
	.....	.....	2.6
20	187	.....	2.8
	137	+	2.6
	312	.....	2.7
	.....	.....	2.3
21	110	+	3.1
	.....	.....	2.5
22	214	.....	.....
	406	+	2.5
	350	.....	2.5
23	175	.....	.....
	220	+	3.2
	420	.....	5.7 *
24	175	+	3.1
25	216	.....	.....
	316	+	3.2
	500	.....	.....
32	50	.....	2.8
	70	+	2.9
	100	.....	3.2
	100	.....	2.8
33	167	.....	.....
	211	.....	.....
	193	+	3.7
	77	.....	.....
	205	.....	.....
37	40	+	2.9
55	200	?	6.0
	218	.....	4.3
	.....	.....	5.3
54	?	?	3.7

* Slightly turbid.

A positive globulin test was a constant finding in these cases, but the quantitative value of this test being only approximate, prevents it from having the same significance as the reduction index.

Other Forms of Meningitis.—The reduction index was determined on the fluids from eight cases of epidemic meningitis. These fluids varied from clear to very turbid. The index was above 3 in all but one fluid, a clear fluid with an index of 2.4. In one case despite a clear fluid, the index remained constantly above 3. A high index may be of value from the standpoint of prognosis, since as pointed out by Mayerhofer, the index in meningococcus meningitis, declines after administration of serum in favorable cases, despite the high reduction value of the serum itself. Two cases of streptococcus meningitis gave indices of 2.7 and 4.8, respectively. The fluid from a patient with pachymeningitis hemorrhagica interna, which was bloody on numerous punctures, and from which the meningococcus was isolated, finally became clear. The fluid showed an occasional red blood cell microscopically. Globulin reaction was positive, cell count 50. The index was 3.4.

Poliomyelitis.—The index was determined on three cases of acute poliomyelitis. The readings varied from 2.5 to 3.3 in the acute stage, showing a decline coincident with the disappearance of acute symptoms.

Serous Meningitis and Encephalitis.—In his original article on the reduction index, Mayerhofer particularly emphasized the value of the test in the differentiation of tuberculous meningitis from serous meningitis, or meningismus associated with infectious diseases. Simon,[4] in a critical article, denied its value in these cases. Simon records the index findings in five patients with meningeal symptoms associated with pneumonia. His readings varied from 2.1 to 2.8. In none of these cases did meningitis develop, all but one clearing up. This particular case on postmortem examination showed no pathological changes in the brain or spinal cord. In a subsequent article, Mayerhofer,[5] answering Simon's criticism, stresses the value of determinations made on separate parts of the same fluid, the fluid in tuberculous meningitis giving a higher index in the latter portion, while the reverse is true in serous meningitis. Furthermore, he stated[6] that in mild serous meningitis, a high index, if obtained, remained so for

4. Simon, G.: Zur Untersuchung des Liquor cerebrospinalis nach Mayerhofer, Wien. klin. Wchnschr., 1911, xxiv, 94.

5. Mayerhofer, E.: Kritische Bemerkungen zur Arbeit von G. Simon über meine Methode der Permanganattitration des Liquor cerebrospinalis, Wien. klin. Wchnschr., 1911, xxiv, 205.

6 Mayerhofer, E., and Neubauer, R.: Ueber Meningitis Tuberculosa und Meningitis serosa, Ztschr. f. Kinderh., 1912, iii, 155.

only a short time. In the malignant cases, he claimed a prognostic value for the high index, illustrated by his Case 119, one of enteritis gravissima in which the index was persistently high. On postmortem examination, a "hyperemia leptomeningum gravis et hyperemia cerebri" was found.

From our experience with the reduction index in such cases, we doubt the advisability of laying down any fast rules in regard to the findings in this condition. Table 2 gives the character of cases we have classified under this heading. The determinations varied from

TABLE 2.—Serous Meningitis and Encephalitis

Case No.	Index	Symptoms	Diagnosis
14	2.3	Pain on flexion of neck. Hyperesthesia. Suggestive Kernig's sign	Septicemia, streptococcic
15	2.3	Marked rigidity of neck. Pain on flexion of head. Positive Kernig's sign	Pneumonia, lobar
27	2.25	Strabismus, rigidity of neck. Tense fontanel. Positive Kernig's sign	Post operative spina bifida
30	2.4	Convulsions. Restlessness. Changed mentality	Tuberculosis of spine and tuberculosis, pulmonary
48	1.8	Meningeal symptoms	Pneumonia, lobar
49	2.9	Marked meningeal symptoms at first reading. Disappearance of these at second reading	Pneumonia, lobar
.....	1.8		
50	2.0	"Meningeal symptoms"	Pneumonia, lobar

very low to moderately high. This variation seems to indicate quite a difference in the severity of what we are pleased to call serous meningitis. While routine cultures from the spinal fluid of some of these cases showed no micro-organisms, it seems quite probable that actual changes do occur in the meninges during so-called meningismus associated with the infectious diseases, which account for the variability encountered in the permanganate reduction index. The work of Rohdenburg[7] seems to refute Quincke's[8] original idea that serous

7. Rohdenburg, G. L., and Vander Veer, A.: The Spinal Fluid in Pneumonia, Jour. Am. Med. Assn., 1915, lxiv, 1227.

8. Quincke, H.: Ueber Meningitis serosa, Samml. klin. Vortr., Leipzig, 1893, p. 655.

meningitis is not associated with infection by micro-organisms. All but one of the cases gave borderline readings. Case 49, at the outset showed marked meningeal symptoms. The cell count was 147, of which 71 per cent. were polymorphonuclears. The globulin reaction was positive. On the decline of meningeal symptoms, the index became 1.8. It seems reasonable to suppose that the meninges in such a patient actually undergo an inflammatory reaction as found by Voisin.[9]

Cases 27 and 30 came to necropsy. The first developed meningeal symptoms a few days following an operation for spina bifida. Rigidity of the neck, strabismus, a tense fontanel, and a positive Kernig's sign were present. A ventricular puncture was done. Twenty cubic centimeters of clear fluid were removed under greatly increased pressure. The cell count was 3, the globulin reaction was negative. Postmortem examination showed a marked edema of the brain, marked internal hydrocephalus and hyperemia of the entire brain.

The second case was one of Pott's disease with pulmonary tuberculosis. A few days after admission, the patient had a severe convulsion, following which irregular twitching of the facial muscles occurred for several days. The patient acted peculiarly, was talkative and restless. On lumbar puncture, 20 c.c. of clear fluid were obtained under pressure. The cell count was 10, globulin negative. Three days later lumbar puncture was repeated with similar findings, except that the Noguchi globulin test showed a faint turbidity. Postmortem examination revealed a hyperemia of the cortex and base of the brain. The French school of pediatricians have noted the great frequency of encephalitis in children, and Lucas has called attention to the occasional similarity of findings in the cerebrospinal fluid of encephalitis and tuberculous meningitis. It is possible that the cases detailed above belong to this class.

In one of the cases of congenital lues which occurred in our series, lumbar puncture was performed following the development of meningeal symptoms. The cell count was 1, the globulin negative, index 2.3. In this instance the actual presence of pathological change was shown only by the index and confirmed by postmortem examination, which revealed an edema of the pia over the entire cortex and base, and an excess of cerebrospinal fluid. On the left side, at the Sylvian fissure, there was an inflammatory exudate. Péhu[10] has recently called attention to the occurrence of encephalitis associated with congenital syphilis, which differs pathologically in no respect from ordinary enceph-

9. Voisin, R.: Maladies des meningès, Paris, 1912, p. 215.

10. Péhu and Gardére: Sur un cas d'encephalite aigüe avec présence du Tréponème du Niveau des lésions, Arch. de méd. d. Enf., 1915, xviii, 330.

alitis. Several similar cases occurred in our series. Clinically they resembled the picture of encephalitis. The indices were either borderline or high readings.

Brain Tumor.—In no other condition was the value of the Permanganate Reduction Index as strikingly demonstrated as in a case of brain tumor observed by one of us. In this case, the index remained persistently low, while from the clinical picture, the diagnosis of tuberculous meningitis, later, that of solitary tubercle, had been made by several able clinicians. The postmortem examination revealed a tumor of the cerebellum.

CASE 1.—*History.*—B. N., aged 2½ years, male, was first seen Feb. 11, 1914. Family history: Father and mother living and well. Grandmother had cough, but was never in contact with the child.

Past History.—Nursed one year. Repeated "colds" all last winter.

Present Illness.—Has lost 7 pounds in the last seven weeks. Has had irregular fever to 101, vomiting "now and then," becoming more marked. Bowels costive; chronic headache; no cough. Routine examination reveals nothing abnormal except emaciation; no rigidity of neck. No Kernig's sign.

Blood Examination: Red cells, 5 million; white cells, 16,000; hemoglobin, 75 per cent.

Feb. 14, 1914. Ophthalmoscopic examination shows choked disk. Lumbar puncture gives opalescent fluid, 330 cells per c.mm., lymphocytes predominant. No micro-organisms on culture or smear preparation. Pirquet definitely positive.

Feb. 17, 1914. Slight rigidity of neck. Suggestive Kernig's sign on left side; not present on right side. Babinski present on left side; not present on right side. Marked ataxia. Romberg in sitting posture. Spinal fluid examination shows same findings as on previous examinations.

Feb. 18, 1914. Fluid not under pressure. Still opalescent. Reduction index 1.7. Physical examination as noted.

Feb. 21, 1914. Fluid not under pressure. Patient better. Trace of blood in cerebrospinal fluid; index 1.8.

Feb. 24, 1914. Fluid clear, no micro-organisms ,index 1.9. Wassermann on fluid weakly positive. Lange test (see Chart, Fig. 1).

Feb. 28, 1914. Fluid clear. Index 1.9. Lange test (Chart, Fig. 2).

March 2, 1914. Fluid clear. Blood serum; Wassermann weakly positive. Lange test (Chart, Fig. 3).

March 9, 1914. Index 1.5. Lange test (Chart, Fig. 4).

March 17, 1914. Index 1.5. Lange test negative in all dilutions.

April 16, 1914. Cerebellar symptoms.

April 18, 1914. Index 1.3. Lange test negative in all dilutions.

May 9, 1914. Clear fluid. Index 1.7.

May 25, 1914. Index 2. Wassermann on fluid negative.

Postmortem Examination.—Anatomical diagnosis, Ependymal glioma. Internal hydrocephalus.

The following case, though lacking the finality of a postmortem examination, bears a resemblance to the case just noted. However, it is quite possible that it is one of solitary tubercle, with low indices, as reported by Mayerhofer.

CASE 2.—*History.*—I. L., girl aged 4½ years, was admitted to the Children's Memorial Hospital, Feb. 4, 1915.

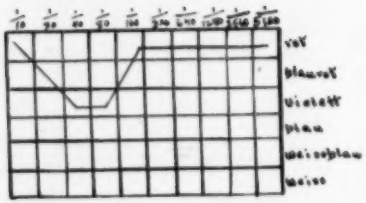

Fig I

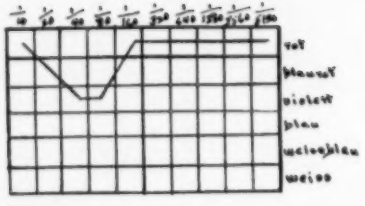

Fig. 2

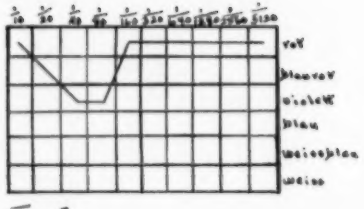

Fig. 3

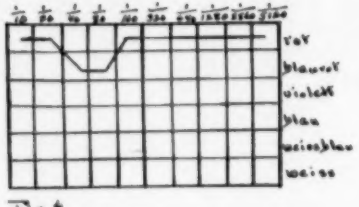

Fig 4

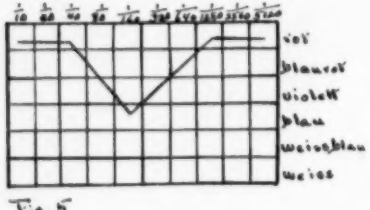

Fig. 5

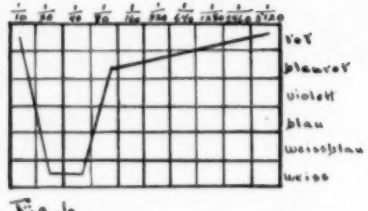

Fig. 6

Figures 1, 2, 3 and 4 refer to the case of B. N. (Case 1)—ependymal glioma—showing the curves of the Lange test. Figures 5 and 6 show curves of Lange test in the case of I. L. (Case 2), of probable tumor.

Family History.—Father and mother living and well; two other children well. No tuberculosis in family. Mother had one miscarriage.

Past History.—Birth and infancy normal. At 6 weeks patient had pertussis followed by pneumonia.

Present Illness.—Patient has been ill since the early part of December, 1914. Vomits once or twice daily. Has no appetite. Fever present for a few days. Occasional chills. Constipated. Strabismus for four weeks. Coughed three weeks ago but not now. Restless at night. Drowsy and stupid during the day. Has lost considerable weight.

Physical Examination.—Patient is a fairly well developed and nourished girl. Answers questions. "Wants to go home." "Wants mamma."

Eyes: Internal strabismus, right eye; pupils alike; react to light and are regular.

Tonsils slightly enlarged.

Heart sounds irregular. Breath sounds normal. Abdomen scaphoid. Liver felt at costal margin. Spleen not felt. No adenopathy. No paralysis. Knee-jerks absent. Brudzinski's sign present. Kernig's sign not present. Slight rigidity of neck.

Blood Examination: Red cells, 5,240,000; white cells, 9,000; hemoglobin, 80 per cent.

Differential Count: Polymorphonuclears, 60 per cent.; small mononuclears, 38 per cent.; large mononuclears, 2 per cent.

Wassermann negative.

Feb. 4, 1915. Lumbar puncture; 30 c.c. clear fluid; cell count 25, mononuclears predominating. Globulin test negative.

Feb. 5, 1915. Lumbar puncture; 5 c.c. clear fluid. Globulin test negative. Index 1.7.

Feb. 6, 1915. Von Pirquet negative.

Feb. 8, 1915. Von Pirquet negative.

Feb. 8, 1915. Ophthalmoscopic examination: Choked disk, both eyes.

Roentgen Examination of Chest: Negative, except for slight mottling at the lung roots.

Lumbar Puncture: Ten c.c. clear fluid; cell count 30; globulin test negative. Index 2. Lange test (Chart, Fig. 5).

Feb. 9, 1915. Sits up all the time. Hides eyes. Seems depressed. No subjective complaints. Knee-jerks present both sides. No Kernig's nor Brudzinski's sign present. Neck is slightly rigid. Walks with slight ataxic gait. No Romberg sign. Babinski's sign present on both sides; left more marked than right. No clonus.

Feb. 15, 1915. Lumbar puncture: 20 c.c. clear fluid; cell count 30; polynuclears and mononuclears equal. Globulin test negative. Index 1.9.

Feb. 19, 1915. Lumbar puncture: 8 c.c. clear fluid. Index 2.

Feb. 23, 1915. Clonus present on right side. Knee-jerk absent on right side. Clonus absent on left side. Knee-jerk increased on left side.

Feb. 24, 1915. Ophthalmoscopic examination shows less choked disk.

Feb. 27, 1915. Condition practically unchanged. Brudzinski's sign present. Knee-jerks present both sides.

March 5, 1915. Lumbar puncture: cell count 16, globulin test negative. No tubercle bacilli demonstrated in any fluids. Fluid centrifugalized and 1½ c.c. of sediment injected intraperitoneally into guinea-pig. Lange test (Chart, Fig. 6).

March 9, 1915. Patient decidedly worse. Marked retraction of head. Vomits frequently. Sighing respiration. Answers when addressed. No change in physical examination. Temperature normal throughout entire stay. Taken home against advice.

Death two weeks later.

Necropsy on guinea-pig performed five weeks after injection. Pig had gained 75 gm. No gross lesions of tuberculosis evident.

Another very similar case, which ended fatally, occurred in our series. The index remained low. Guinea-pig injection with the spinal fluid was negative. The patient having been taken home against advice one day before death, prevented postmortem examination.

Table 3 gives a variety of conditions associated with convulsions occurring in our series. The low index values occurring in these cases are self-explanatory.

TABLE 3.—FLUIDS FROM PATIENTS WITH DISEASES OTHER THAN MENINGITIS

Case	Diagnosis	Index
8	Cystitis	1.6
9	Little's disease	2.0
10	Tetany	1.3
18	Otitis media	2.1
19	Convulsions (undiagnosed)	1.9
26	Convulsions (undiagnosed)	2.1
34	Cerebral syphilis	1.9
35	Amaurotic family idiocy	1.9
51	Meningismus (undiagnosed)	1.6
52	E. B. (no meningeal symptoms)	1.6

CONCLUSIONS

The permanganate reduction index of Mayerhofer was determined on the spinal fluids obtained from patients admitted to the hospital for a number of different conditions. Indices classify themselves into three groups:

1. Low indices, below 2.
2. Borderline indices, between 2 and 2.5.
3. High indices, above 2.5.

All normal fluids or fluids obtained from patients presenting convulsions or other meningeal symptoms without actual inflammation of the brain or meninges give low indices. A few such conditions are tabulated in Table 3. Such an index may be obtained in brain tumor.

Borderline indices may occur in the early stage of an inflammatory process, involving the brain or meninges, in serous meningitis, encephalitis or other conditions associated with hyperemia of the brain. High indices, if constant, almost invariably indicate an actual inflammatory process of the brain or meninges. The two diseases most likely occurring with such indices are tuberculous meningitis and acute poliomyelitis. The Mayerhofer test possesses a distinct value in the examination of the cerebrospinal fluid.

TREATMENT OF TYPHOID FEVER BY INTRAVENOUS INJECTIONS OF POLYVALENT SENSITIZED TYPHOID VACCINE SEDIMENT

STUDIES IN TYPHOID IMMUNIZATION VI *

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Since the work of Fraenkel[1] in 1893, killed preparations of the typhoid bacillus have been injected subcutaneously as a means of treatment in typhoid fever. Little interest was at first awakened by the suggestive results of Fraenkel except in a discussion of the specificity of his treatment (Rumpf,[2] Kraus and Buswell,[3] Presser.[4]) In 1902 Petruschy[5] used a combination of vaccine and immune serum in typhoid, and in 1908 Pescarolo and Quadrone[6] advocated the use of a living, avirulent typhoid culture. Following the interest in vaccine therapy awakened by Wright, increasingly frequent reports on the possible value of typhoid vaccines in typhoid fever have appeared. In 1912 Callison[7] summarized the results obtained by numerous authors, chiefly English and American, in 747 cases, and in 1915 Krumbhaar

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1. Fraenkel, E.: Ueber spezifische Behandlung des abdominal Typhus, Deutsch. med. Wchnschr., 1893, xix, 985.

2. Rumpf, T.: Die Behandlung des Typhus abdominalis mit abgetodteten Cultures des bacillus Pyocyaneus, Deutsch. med. Wchnschr., 1893, xix, 987.

3. Kraus, F., and Buswell, H. C.: Ueber die Behandlung des Typhus abdominalis mit abgetodteten Pyocyaneus Culturen, Wien. klin. Wchnschr., 1894, vii, 511, 595.

4. Presser, L.: Ueber die Behandlung des Typhus abdominalis mit Injektionen von Culturflüssigkeiten von Bac. Typhi und Bac. Pyocyaneus, Ztschr. f. Heilk., 1895, xvi, 113.

5. Petruschy, J.: Spezifische Behandlung des abdominal Typhus, Deutsch. med. Wchnschr., 1902, xxviii, 212.

6. Pescarolo, B., and Quadrone, C.: Aktiv Immunisation durch subcutane Injektionen lebenden Typhus bazillen bei Eberthscher Infektion, Zentralbl. f. inn. Med., 1908, xxix, 989.

7. Callison, J. G.: The Therapeutic Use of Vaccines in Typhoid Fever, Am. Jour. Med. Sc., 1912, cxliv, 350.

and Richardson[8] could collect over 1,800 cases reported on by forty authors. It is known that many physicians, and probably an increasing number of them, use vaccines in typhoid because they find they do no harm and they believe them to do some good. The best studied groups of cases treated in this manner, however, give ground for little unrestrained enthusiasm, and certainly no claim that any such type of specific therapy has been attained as is the case in diphtheria and epidemic meningitis. The best that may be said is that the ordinary type of killed typhoid vaccine administered subcutaneously in controlled groups of cases may cause a shortening of the course of the disease, a lower mortality, and perhaps fewer relapses and complications. We purposely refrain from further analysis of the ordinary type of vaccine therapy which has been described, since we are to deal with methods and results which we believe constitute a new era in the specific treatment of typhoid fever.

In the last few months reports on the intravenous injection of ordinary heat-killed vaccine (Thiroloix and Bardon,[9] Kraus and Mazza[10]) and particularly on the intravenous injection of sensitized vaccines (Ichikawa[11]) in typhoid fever, have led us to anticipate a far more hopeful future in combating this disease than has hitherto seemed likely. Before proceeding to a consideration of the results recorded by several authors in actual cases, we may be pardoned for repeating the experimental evidence by which we were led to a practical consideration of this problem before most of these observations were made.

In a previous article in this series Gay and Claypole[12] described a new and specific form of hyperleukocytosis which occurs in immunized rabbits on intravenous reinjection of the specific antigen (bacteria, red blood cells, serum). We found, for example, that the injection of living typhoid bacilli, or of typhoid vaccine in a typhoid immune rabbit, caused the leukocytes to fall in the first two or three hours and then to rise to critical levels that occurred at about the twelfth and again at the twenty-eighth hour. At the latter period leukocyte counts of 150,000 per cubic millimeter were not uncommon, a surprising rise from the normal count of from 8,000 to 12,000, and one that has not been

8. Krumbhaar and Richardson: The Value of Typhoid Vaccine in the Treatment of Typhoid Fever, Am. Jour. Med. Sc., 1915, cxlix, 406.

9. Thiroloix and Bardon: Vaccin Typhique intraveineux, Centralbl. f. Bakt. Ref., 1914, lx, 212 (3d ref.).

10. Kraus, R., and Mazza, S.: Zur Frage der Vakzinetherapie des Typhus abdominalis, Deutsch. med. Wchnschr., 1914, xl, 1556.

11. Ichikawa, S.: Mitteil. d. med. Gesellsch., zu. Osaka, April, 1912, x, No. 5; Abortive Treatment of Typhoid and Paratyphoid, Sei-I-Kwai Med. Jour., Tokyo, 1914, xxxiii, No. 12, p. 73, or Ztschr. f. Immunitätsforsch., 1914, xxiii, 32.

12. Gay, F. P., and Claypole, E. J.: Specific Hyperleukocytosis: Studies in Typhoid Immunization, IV, THE ARCHIVES INT. MED., 1914, xiv, 662.

attained, so far as we are aware, by other experimental methods. These hyperleukocytic crises, moreover, are coincident with the destruction of the typhoid bacillus in the immune animal, and would appear to be the cause of it. The same dose of typhoid bacilli in a normal rabbit produces a distinct but markedly lower grade of hyperleukocytosis. We logically regarded the extreme grade of hyperleukocytosis as dependent on the presence of antibodies (tropins) in the immune animal, an hypothesis that we were able to verify by obtaining similar results in normal rabbits by the use of tropinized (sensitized) cultures of *B. typhosus;* that is to say, cultures that had been treated with an immune serum. From these experiments it seemed reasonable to express the opinion that the intravenous injection of sensitized typhoid vaccine offered a possibly successful method of treating typhoid fever.[13] As a preliminary to the intended application of the sensitized typhoid vaccine sediment, which we advocate for prophylactic use against typhoid fever,[14] in the treatment of this disease, we tested its curative effect in rabbits that had been made "carriers" of the typhoid bacillus.[15] A few of these animals were apparently freed of the typhoid bacillus by the intravenous injection of the sensitized vaccine; that a majority of them remained unaffected is due to the inaccessibility of the microorganism in the gallbladder of such carrier rabbits. Further preliminary experiments have shown us that relatively large doses of sensitized typhoid vaccine can be given with safety directly into the circulation of rabbits and monkeys, and even when these animals have been partially immunized against *B. typhosus.* The latter condition, owing to the presence of agglutinins, was aimed to simulate the condition in typhoid fever.[16]

On the basis of this experimental evidence accumulated in 1913 and 1914, we were prepared to attempt treatment of human cases, but no opportunity presented itself until early in 1915. Since February of this year we have been privileged to examine over 100 cases of clinically suspected typhoid, to carry out in most of them our own laboratory examinations, and to cooperate in the treatment of the great majority of them in which a diagnosis of typhoid fever could be fully confirmed.

13. Gay, F. P., and Claypole, E. J.: Specific Hyperleukocytosis: Studies in Typhoid Immunization, IV, THE ARCHIVES INT. MED., 1914, xiv, 669.

14. Gay, F. P., and Claypole, E. J.: An Experimental Study of Methods of Prophylactic Immunization Against Typhoid Fever: Studies in Typhoid Immunization, V, THE ARCHIVES INT. MED., 1914, xiv, 671.

15. Gay, F. P., and Claypole, E. J.: The "Typhoid Carrier" State in Rabbits as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus: Studies in Typhoid Immunization, I, THE ARCHIVES INT. MED., 1913, xii, 613.

16. It may be of interest to note in this connection that efforts made to produce typhoid carriers in Macacus rhesus monkeys by injecting large doses of living culture directly into the circulation have failed.

This study has been made possible by the hearty interest and cooperation of over fifty physicians in Berkeley, Oakland, San Francisco and their environs.[17]

We may now proceed to a brief discussion of the results of other investigators who have made use of the recent innovations in the vaccine treatment of typhoid, leaving analysis of details of method and result for comparison with our own records. Recent results with subcutaneous injection of unsensitized vaccine are purposely omitted, however favorable.

Sensitized Typhoid Vaccines Employed Subcutaneously.—Besredka[18] suggested in 1902 the use of dead or living bacteria that had previously been treated with an immune serum, the excess of which was subsequently removed, as means of producing active immunity without severe reaction. In 1911 Metchnikoff and Besredka[19] on the basis of experiments on anthropoid apes, advocated the use of living sensitized typhoid vaccines as the best prophylactic against typhoid fever and their subsequent results[20] would seem to indicate that they are as good or better than the ordinary vaccine for this purpose. These living sensitized cultures have also been employed subcutaneously in the treatment of typhoid fever during the last few years by several French observers. We find reports by Ardin-Delteil, Negre and Raynaud,[21] Boinet,[22] Deléarde and Leborgne,[23] Sablé,[24]

17. We wish to express our deepest appreciation to the following physicians who have placed cases at our disposal for this study: Drs. E. N. Ewer, Dudley Smith, George L. Reinle, C. A. DePuy, E. M. Lundegaard, C. A. Queirolo, A. Liliencrantz, Guy H. Liliencrantz, M. L. Emerson, A. F. Clarke, W. H. Irwin, E. A. Majors, H. A. Mackinson, A. M. Shade, C. S. Powell, C. H. Rowe, E. G. Simon, C. R. Krone, F. M. Sylvester, A. S. Kelly, J. M. Shannon, W. H. Streitmann, E. von Adelung and P. F. Abbott of Oakland; Frank W. Simpson, May H. Sampson, Robert Hector, A. M. Meads, L. A. Martin, F. H. Van Tassell, J. J. Benton, J. M. Beuckers, H. S. Delamere, M. F. Toner, R. Paroni, R. T. Legge, H. W. Crane, Sheffield; W. A. Wood, T. C. McCleave of Berkeley; J. K. Hamilton, R. E. Burns and H. M. Pond of Alameda; H. C. Miller, C. A. Wills of San Leandro; H. C. Moffitt, Wm. P. Lucas, Geo. E. Ebright, P. K. Brown, Chas. A. Pauson, Fred G. Burrows, R. L. Wilbur, G. H. Evans, and R. D. MacKinnon, San Francisco; and L. L. Stanley, San Quentin.

18. Besredka, A.: De l'immunisation active contre la peste, le cholera et l'infection typhique, Ann. d. l'Inst. Pasteur, 1902, xvi, 918.

19. Metchnikoff, E., and Besredka, A.: Réchérches sur la fiévre typhoide experimentale, Ann. de l'Inst. Pasteur, 1911, xxv, 193; Des vaccinations antityphiques, Ibid., p. 865.

20. Metchnikoff, E., and Besredka, A.: Des vaccinations antityphiques, Ann. de l'Inst. Pasteur, 1913, xxvii, 597.

21. Ardin-Delteil, Negre, L., and Raynaud, Maurice: Sur la vaccinotherapie de la fiévre typhoide, Compt. rend Acad. d. sc., 1912, clx, 1174; Recherches sur les reactions humorales des malades atteints de fiévre typhoide traités par le vaccin de Besredka, Compt. rend Soc. de biol., 1913, lxxiv, 371.

22. Boinet: Vaccinotherapie de la fiévre typhoide par le virus sensibilisé de Besredka, Compt. rend. Soc. de biol., 1913, lxxiv, 507.

23. Delearde and Leborgne: Province méd., June 21, 1913, p. 273.

24. Sablé: Jour. d. sc. méd. de Lille, July, 1913, No. 38, p. 25; No. 29, p. 49.

Netter,[25] Roques[26] and Alfaro,[27] Feistmantel[28] and Garbat[29] have employed other preparations of killed sensitized vaccines. In these, as in the later cases, the criteria of improvement differ with individuals and are hard to compare.

Unsensitized Vaccine Administered Intravenously.—Several different preparations of killed typhoid vaccine have been tried intravenously but Vincent's polyvalent ether-killed autolysate has been used in the greatest number of cases. Following the brief communication of Thiroloix and Bardon[9] in 1913, articles have appeared by Kraus and Mazza,[10] Kraus,[30] Biedl,[31] Csernel and Marton,[32] Rhein,[33] Reibmayr,[34] Mazza,[35] Holler,[36] Löwy, Lucksch and Wilhelm,[37] Paulicek,[38] Ditthorn and Schultz[39] and McWilliams.[40] This intravenous injection of typhoid vaccine gives a definite reaction which if certain limits of dosage are exceeded may be alarming or dangerous. We shall consider this reaction in more detail later.

Sensitized Vaccine Administered Intravenously.—So far as we are aware, three types of sensitized typhoid vaccine have been employed

25. Netter: Bull. et mém. Soc. méd d. hôp., July 24, 1913, p. 126.

26. Roques: Contribution a l'étude de la vaccinotherapie de la fiévre typhoide par le virus-vaccin sensibilisé antityphique vivant de Besredka, Thèses University of Toulouse, 1913, Ed. Ch. Dirion.

27. Alfaro, A.: Rev. Soc. Med. Argentina, 1913, p. 683.

28. Feistmantel, C.: Ueber Prophylaxie und Therapie des Typhus Abdominalis mittels Impfstoffen, Wien. klin. Wchschr., 1915, xxviii, 230.

29. Garbat, A. L.: Sensitized Versus Nonsensitized Typhoid Bacteria in the Prophylaxis and Treatment of Typhoid Fever, Jour. Am. Med. Assn., 1915, lxiv, 489.

30. Kraus, R.: Bemerkungen ueber Schutzimpfungen und eine Bakteriotherapie des Typhus abdominalis, Wien. klin. Wchnschr., 1914, xxvii, 1443.

31. Biedl, A.: (Letter to Paltauf) Zur Vakzinetherapie des Typhus abdominalis, Wien. klin. Wchnschr., 1915, xxviii, 125; Therapeutische Verwendung von Typhus-Impfstoffen beim Menschen, Prag. med. Wchnschr., 1915, xl, 53.

32. Csernel, E., and Marton, A.: Die Therapie des abdominal Typhus mit nicht sensibilisieter Vakzine, Wien. klin. Wchnschr., 1915, xxviii, 229; Die Behandlung des typhus abdominalis mit nicht sensibilisieter Vakzine, Ibid., p. 733.

33. Rhein, M.: Zur Bakteriotherapie des Typhus abdominalis, München. med. Wchnschr., 1915, lxii, 427.

34. Reibmayr, H.: Ueber Impfstoffbehandlung des Typhus abdominalis auf intravenösem Wege, München. med. Wchnschr., 1915, lxii, 610.

35. Mazza, S.: Die Bakteriotherapie des Typhus abdominalis, Wien klin. Wchnschr., 1915, xxviii, 64.

36. Holler, G.: Zur Vakzinetherapie des Typhus abdominalis, Ztschr. f. klin. Med., 1915, lxxxi, 462; Erfahrungen über Bakteriotherapie des Typhus abdominalis, Med. klin., 1915, xi, 639 and 668.

37. Löwy, R., Lucksch, F., and Wilhelm, E.: Zur Vakzinetherapie des Typhus abdominalis, Wien. klin. Wchnschr., 1915, xxviii, 756.

38. Paulicek, E.: Zur Frage der Typhusheilimpfungen, Wien. klin. Wchnschr., 1915, xxviii, 759.

39. Ditthorn, F., and Schultz, W.: Zur Antigenbehandlung des Typhus, Med. Klin., 1915, xi, 100.

40. McWilliams, H. I.: Treatment of Typhoid Fever with Typhoid Vaccine Administered Intravenously, New York Med. Rec., Oct. 16, 1915, p. 648.

for intravenous administration in typhoid fever. Ichikawa,[11] who began this type of treatment (1913), used recent cultures of *B. typhosus* sensitized by the serum of patients recovering from typhoid fever and killed, or at least attenuated, by the addition of phenol. A similar method has been employed by Koranyi.[41] A considerable series of cases have since been treated by Biedl,[31] Eggerth,[42] Sladek and Kotlowski,[43] Boral,[44] Holler,[76] Löwy, Luksch and Wilhelm,[37] and F. Meyer,[45] who, for the most part, have employed the Besredka living sensitized vaccine.

Any comparison of the results of treatment by these three most recent methods on the basis of percentage benefited, is at best only of suggestive value. As already stated, the criteria on which an estimation of benefit is based vary and no absolute standard is possible, nor do mortality figures offer any conclusive results, but they may be added for completeness since they are at least more definite than any estimated benefit. Since many of the deaths in typhoid are, properly speaking, accidental (hemorrhage, perforations) they cannot be used correctly as criteria of the efficiency of treatment, particularly in those patients that are treated fairly late in the disease where such evolving lesions could not reasonably be affected.

TABLE 1.—SUMMARY OF RECENT CASES OF TYPHOID FEVER REPORTED AS TREATED BY NEWER METHODS OF VACCINE TREATMENT

	Authors	Number Cases	Benefited Per Cent.	Mortality Per Cent.
1. Cases treated by subcutaneous injection of sensitized vaccine....	11	253	57. †	7.1
2. Cases treated by intravenous injection of untreated vaccine....	14	259	63.	19. *
3. Cases treated by intravenous injection of sensitized vaccine....	8	207	81.8	9.6

* Due in part to inclusion of a large number of war cases by Paulicek[39] where delayed transportation from the front increased mortality.

† Of 201 cases.

This summary would suggest that the best method of treatment of the three would be the intravenous injection of living sensitized vaccine as based on a higher percentage of benefit and lower mortality than the intravenous injection of unsensitized vaccine. Individual reports would

41. Koranyi, A. V.: Zur Vakzinebehandlung des Typhus abdominalis, Wien. klin. Wchnschr., 1915, xxviii, 85.

42. Eggerth, H.: Zur Vakzinetherapie des Typhus abdominalis, (Letter published by Paltauf), Wien. klin. Wchnschr., 1915, xxviii, 126.

43. Sladek, J., and Kotlowski, St.: Zur Vakzinetherapie des Typhus abdominalis, Wien. klin. Wchnschr., 1915, xxviii, 389.

44. Boral, H.: Beitr. zur Frage der Typhustherapie mit Besredka-Vakzine, Wien. klin. Wchnschr., 1915, xxviii, 415.

45. Meyer, F.: Spezifische Typhusbehandlung, Berl. klin Wchnschr., 1915, lii, 677.

indicate that this difference is really more marked than the summary would show, owing to the fact that the benefit in the third category is more likely to be in the nature of an abortive cure with critical fall of temperature rather than a gradual amelioration and lysis of the fever. Moreover, several authors who have used two or more of the methods do not hesitate unanimously to favor the intravenous over the subcutaneous method and sensitized vaccine over the unsensitized. (Holler,[36] Thiroloix and Bardon,[9] Biedl,[21] Meyer,[45] Löwy, Lucksch and Wilhelm.[37])

PERSONAL CASES

Owing to the irregular conditions under which our cases occurred in the hospital or home practice of a number of different physicians, it has been necessary for our own records to esablish the diagnosis to our own satisfaction by a uniform method. It has also been a pleasure to be able to cooperate in a relatively large number of cases in the differential diagnosis of typhoid fever from other infections, on the basis of laboratory examinations. Although there is no need of defending the value of laboratory methods as aiding in the diagnosis of typhoid fever, it may still be pertinent to express the value of a complete series of negative examinations as tending to exclude typhoid even in the presence of a temporary clinical appearance of the disease. This is particularly important in view of the fact that the laboratory diagnosis in general practice depends almost entirely on a Widal reaction alone, and that often performed in a not wholly accurate manner. We wish, incidentally, therefore, to contribute our laboratory results in the differential diagnosis of typhoid, before considering the treatment of those cases in which the diagnosis was positive.

We have carried out laboratory examinations on 105 cases of suspected typhoid fever which may be subdivided as follows:

Total number of cases examined	105
Cases of proved typhoid	65
Cases of typhoid not included as treated	12
Cases treated as suggested	53

Method of Laboratory Examination.—With few exceptions in the earlier part of the investigation, the routine laboratory examination of each case has been as follows:

1. Blood Culture.—Ten c.c. of blood, taken from a vein at the elbow, after proper preparation of the skin with iodin, are mixed with 200 c.c. of 10 per cent. bile broth. In a number of cases the relative intensity of the bacteriemia was estimated by plating 1 or 2 c.c. in melted agar (10 c.c.). The broth cultures were examined daily to the tenth day or until positive. Organisms of correct morphology for the typhoid bacillus were identified by the usual cultural methods in several sugars (glucose and lactose particularly) and in litmus milk. Final diagnosis of a suspected organism depended on agglutination by an antityphoid serum of high potency.

2. Widal.—Two to 5 c.c. of blood were placed in a sterile conical centrifuge tube and allowed to clot. By centrifugalization the serum could then soon

be separated and freed from the blood cells. A series of dilutions beginning 1:10 in a total volume of 1 c.c. of saline was then made running usually to 1:640 or higher when indicated. To each tube there was then added a drop of a thick standard saline suspension of a stock strain of *B. typhosus* grown on agar bottles in large amount and killed and preserved by the addition of 0.1 per cent formaldehyd (40 per cent.). A positive reaction is present in a few hours at room temperature in the lower dilutions, but the final results are read from complete sedimentation of the bacteria with clear supernatant fluid on the following day. This type of macroscopic test gives no pseudo reactions even in a dilution of 1:10 and very rarely to a prezone of inhibition. This low dilution therefore may be regarded as diagnostic of typhoid fever (except in vaccinated cases).

In the few cases in which both Widal and blood culture were negative, the typhoid bacillus was sought for in stools and urine.

Method of Isolation of B. Typhosus from Stools and Urine.—The well known method of smearing direct from stools on successive litmus lactose agar plates was employed. After incubation, suspicious transparent blue colonies were then transferred to agar and sub-cultures made on glucose and lactose media and on litmus milk. Agglutination tests with anti-typhoid serum and paratyphoid "a" and "b" serum of known titer were then made. Enriching with 10 per cent. bile broth was also tried, a loopful of the liquid part of the stool or 1 c.c. of urine being added to 5 c.c. of bile broth. Subsequently plates were made as before.

Cases of Proved Typhoid.—Of the sixty-five cases accepted as typhoid on the basis of laboratory examination, the Widal was positive in sixty (93.7 per cent.), including cases in which the examination was made as early as the fifth day. Of blood cultures taken in fifty-eight of these cases there were forty positive (70 per cent.) including cases taken as late as the thirty-second day.

In One Case Only of this Series were Both Widal and Blood Culture Negative.—In this case the diagnosis was based on the presence of *B. typhosus* in the stools.

In this series of sixty-five cases are included two cases of infection due to *B. paratyphosus* "b," one of which was treated and one of which refused treatment by the method to be described.

Cases of Proved Typhoid That Were Not Treated.—There are twelve cases among the sixty-five of proved typhoid that were not included among the treated patients for the following reasons:

	Cases
No records obtainable from physician	3
Patients died before laboratory diagnosis was complete	2
Post typhoid osteomyelitis	1
Temperature beginning to fall	2
Refused treatment	1
Intravenous injection could not be given	2
Still under treatment	1

These cases are mentioned to emphasize the fact that no choice was exercised in the cases treated. *In every case in which the diagnosis was certainly typhoid the patient was treated if possible except in the*

instances in which a falling temperature might lead to an unwarranted conclusion of benefit produced by the treatment.

There remain forty patients examined in which the clinical diagnosis of suspected typhoid could not be confirmed by laboratory examinations. It is of interest to note that none of these patients gave a positive Widal in dilution of 1 : 10 by the method described. The blood cultures were negative in all. In five of the clinically more promising cases the stools and urine were also negative. From the point of view of the accuracy of the laboratory examinations it is of interest to note that on the basis of further laboratory examinations and their clinical course, thirty-six could be excluded as definitely not typhoid. From their interest in the matter of differential diagnosis it may be of value to catalogue these cases as follows:

	Cases
Malaria	6
Tuberculosis	4
Brief unknown infections	4
Respiratory infections	4
Trichinosis	2
Influenza	2

and one each of the following: Infection following abortion, erysipelas, tonsillitis, measles, prostatitis, postoperative infection of the nose, appendicitis, pleurisy, coryza, pharyngitis, normal, endocarditis, cystitis, constipation.

There remain then four cases which, on the basis of excellent clinical evidence alone and in absence of confirmatory laboratory proof, may still from one point of view be classed as typhoid. These cases are not included in our treated series on the ground of not being certainly proved typhoid. It may be remarked, however, that two of these patients were treated and both *recovered abruptly* with a single intravenous injection of our vaccine. They would, therefore, if added to our series, simply increase the percentage of favorable results.

We have then a series of fifty-three cases of typhoid fever in which the clinical diagnosis was fully verified by laboratory examination and in which it was possible to carry out specific treatment as intended. Owing to the extremely variable conditions under which these cases have been studied, both in hospitals and homes, it has been manifestly impossible in many instances to carry out as complete examinations from the laboratory point of view as could be desired. The general conduct of the cases from the point of view of diet, hydrotherapy and other palliative measures, we have not been able, or, indeed, wished to control. The variations, then, from the standpoint of general treatment are precisely those which one might expect from the logically different conceptions of the disease found in any group of fifty physicians. The

conduct of the vaccine treatment has, however, owing to the uniform courtesy of those who have allowed us the privilege of this study, been developed from our own experience and the interpretations are again our own. We can only feel that if a similar group of cases could be studied and treated under a uniform set of conditions, that the undoubtedly beneficial results which we attribute to this specific treatment would only be enhanced.

METHOD OF TREATMENT

The typhoid vaccine we have used in the treatment of these cases of typhoid fever is one that has already been advocated for the prophylactic immunization of human beings by Gay and Claypole.[14] It consists of the ground sediment of a mixed polyvalent vaccine that has been sensitized by an antityphoid serum and then killed and precipitated by alcohol. From this ground culture the endotoxins are extracted by carbolated saline solution and the remaining sediment of bacterial bodies alone used. On the basis of experimental results this vaccine was found to be superior for protecting rabbits against infection with the typhoid bacillus as compared with other types of vaccine commonly employed, including Besredka's living sensitized vaccine. In the practical use for prophylactic purposes of this vaccine in man it has been shown that it produces less symptomatic disturbance on inoculation (Force[46]) and may be regarded as more protective against typhoid fever in civil communities than several other types of commercial vaccine (Sawyer[47]) as judged by the incidence of typhoid fever among the vaccinated. That the protection afforded by this vaccine employed in civil communities is relative and not so perfect as that hitherto obtained in the United States Army we do not regard as proof that this vaccine may not be as superior to other types of vaccine, including Army vaccine, in practical civil conditions of human infection, as our experimental results in animals would lead us to expect.

This polyvalent, sensitized typhoid vaccine sediment is administered for prophylactic purposes in doses of a suspension of 1/10 mg. of dried bacteria, which corresponds to an original bacteria count of about 750 millions. Such a dose when given subcutaneously in a normal individual very rarely produces more than a slight local reaction. For therapeutic purposes we have given doses intravenously ranging from 1/100 to 1/10 mg., most of the doses being between 1/50 and 1/25 mg. (150 to 300 million bacteria). Such a dose when administered intravenously produces a series of distinct symptoms which vary

46. Force, J. N.: Institutional Vaccination Against Typhoid Fever, Am. Jour. Pub. Health, 1913, iii, 750.

47. Sawyer, W. A.: The Efficiency of Various Antityphoid Vaccines, Jour. Am. Med. Assn., 1915, lxv, 1413.

markedly in intensity with individuals and which are apparently similar in normal and typhoid cases. Although our symptoms are like those described by recent writers who have used the intravenous method for either sensitized or unsensitized dead cultures, it is reasonable to assume that in aliquot parts our vaccine should be less toxic owing to the abstraction of the endotoxins from the ground bacteria. This lesser toxicity seems to have been proved by our results. Although the upper range of doses mentioned may provoke alarming symptoms in the more susceptible individuals, it may be stated at once that in over 150 doses which we have administered no eventual or even temporary harm seems to have been done to the patient. It seems necessary to produce a moderate reaction to bring about the desired results. Before discussing the symptoms that have been noted we may present the record of a typical *severe* reaction produced in a normal individual who volunteered for the purpose.

S. B. H., male, aged 27, had never had typhoid fever or been vaccinated against typhoid. In good health. Oct. 28, 1915, given 180 million sensitized vaccine intravenously.

Leukocyte count before the injection, 6,600; polymorphonuclears, 54 per cent.; lymphocytes, 28 per cent.; large mononuclears, 18 per cent. Temperature, 100 F.

One half hour after injection patient had a general shaking chill lasting thirty minutes. Patient was not cyanotic but had slight respiratory distress due to muscle spasm. Patient vomited twice after chill was over. Temperature rose gradually to a maximum of 104.4 three hours after injection and then fell to 102.4 at the fifth hour and 97.6 at the tenth hour. It had returned to 99.2 in twenty hours, where it remained. Pulse rose to 120.

Leukocyte counts following injection were as follows:

Hours After	No.	Polys. Per Cent.	Lymphs. Per Cent.	Large Monos.	Eosin.
One	4,200	70	22	8	..
Three	7,900	88	6	6	..
Five	8,800	89	6	5	..
Ten	12,000	80	10	10	..
Twelve	12,200	75	11	14	..
Fourteen	15,400	71	16	13	..
Sixteen	16,600	52	15	32	1
Eighteen	14,000	74	8	18	..
Twenty	12,000	60	17	23	..
Twenty-two	11,000	50	6	44	..
Thirty-six	11,600	25	22	52	1
Sixty	11,000	42	27	31	..

This reaction in a normal individual is similar to the one produced in a case of typhoid fever, though distinctly more severe than the average.

A composite picture of the train of symptoms that follows the intravenous injection of our sensitized vaccine given in proper dosage is as follows:

A chill occurs beginning in one half hour to one hour and lasting from a few minutes to ten or fifteen. This chill is accompanied by a rise in temperature of 1 to 3 degrees, which reaches its height within three hours after injection, and then falls. There may be a rise of temperature without chill. The rise in temperature is accompanied by a leukopenia as low as 2,000 to 3,000 per cubic millimeter, which may be preceded by a very transitory hyperleukocytosis during the chill. The chill is accompanied by an increase in the pulse rate (say to 120). Slight cyanosis, slight respiratory distress and frequently discomfort may occur.

The fall in temperature reaches normal or subnormal (as low as 94 F. per rectum) in about twelve hours. This fall in temperature is accompanied by sweating, which may be profuse and last for several hours, relaxation, and usually general amelioration of such symptoms as headache, delirium and the like. The patient often feels perfectly well and demands food and even when this condition is transitory it would seem to be beneficial. Coincidently there is a rise in leukocytes which may reach as high as 40,000 and frequently 15,000 to 20,000. This is represented by a relative polymorphonuclear increase of 80 to 90 per cent. This hyperleukocytosis is the more striking in view of the characteristic leukopenia of typhoid. This particular reaction, which we regard as of peculiar significance, was predictable from our experimental results in rabbits and mentioned in a preliminary communication.[48] It has since been touched on by Holler[36] and confirmed fully by McWilliams.[40]

In four instances we feel that the dose administered was too large for the individual case and in such instances there may be vomiting, cyanosis and even symptoms of collapse, with irregular heart action. In one case in which 800 million were given by another's mistake, there was partial collapse and small hemorrhages in the mouth reported.

The possible dangers of the intravenous method of treatment, whether with unsensitized or with sensitized vaccines, have been mentioned by a number of recent authors. It should be stated that unfavorable reports are based on the use of vaccines more toxic than the one we have employed and the criticisms when severe have been based on a very limited experience. Thus Boral[44] had a single patient who died three days after intravenous injection of the Besredka vaccine, in which case the outcome was attributed, for no specific reason, to the vaccine. Sladek and Kotlowski[43] urge caution owing to possible danger of collapse. Deutsch[49] had one patient who died with meningeal

48. Gay, F. P.: Abortive Treatment of Typhoid Fever by Sensitized Typhoid Vaccine Sediment, Jour. Am. Med. Assn., 1915, lxv, 322.

49. Deutsch, F.: Zur Vakzinebehandlung des Typhus abdominalis, Wien. klin. Wchnschr., 1915, xxviii, 810.

symptoms five days after treatment. Biedl[31] noted increase of epistaxis in two of his patients. Csernel and Marton[32] believe the intravenous injections are contraindicated in hemorrhage, perforation, cholecystitis and cases with irregular heart action. It seems evident that with growing experience the danger decreases (Koranyi[41]) and we are inclined to believe with Holler[30] that the danger lies not so much in the method as in the physician who administers the vaccine. Of the authors mentioned, it is interesting to note that only one (Deutsch) really abandoned the intravenous route.

It would seem evident from the symptoms that we have described that great caution should be used in choosing the dose to be employed with particular regard to the existence of such existing complications as abnormal cardiac functioning and hemorrhage. We have not seen any contraindication in the presence of slight bronchial or bronchopneumonic involvement and have proceeded cautiously with the treatment in four such cases. We have further treated one patient in whom hemorrhage had begun without increasing it.

No detailed method of procedure can be prescribed in treating a given case of typhoid fever by our sensitized sediment. The best results seem to be obtained by provoking a distinct but not too severe reaction of the type outlined, characterized particularly by a temperature excursion and hyperleukocytosis. The dose necessary to produce such a result varies markedly with the individual and with the particular balance already established between the infecting agent and resisting host. A single injection may be all that is necessary to restore the individual to an essentially normal condition as judged from the temperature chart; and as has been mentioned, the subjective symptoms follow the fever. The temperature may drop to normal following the initial rise and remain there, in which case no further injections are necessary, except perhaps to prevent relapse. As a rule, however, the most favorable type of rapid return to normal is a matter of two or three days instead of twenty-four hours, and we have usually waited this longer period before repeating the treatment.

We had best leave further discussion of the variations in method of treatment until we have given our results as a whole, together with such correlations as present themselves between the results produced and the blood findings before and after injection.

Our cases may be readily divided in respect to results, into three rather sharply defined groups, which we herewith exemplify by the appended type charts. (Figs. 1-3).

Group 1. Relatively Unaffected Cases.—This group comprises eighteen, or 34 per cent. of our cases. Each and all of the successive treatments in these cases, although frequently resulting in temperature

excursion, hyperleukocytosis, and the other symptoms, apparently produce no permanent result so far as shortening the course of the disease is concerned. It is not strictly speaking exact to classify these as "unaffected" for, as mentioned, even the temporary ameliorations in temperature and subjective symptoms we are inclined to regard as beneficial. In a number of these cases it will be seen from the remarks on Table 2 that the fever ran permanently lower after treatment. In other cases the bacteria were diminished or disappeared from the blood. It is, moreover, distinctly to be noted that in none of these cases did the use of vaccine apparently weaken the patient or contribute in any demonstrable way to a fatal outcome when such occurred. (Fig. 1, Case 47).

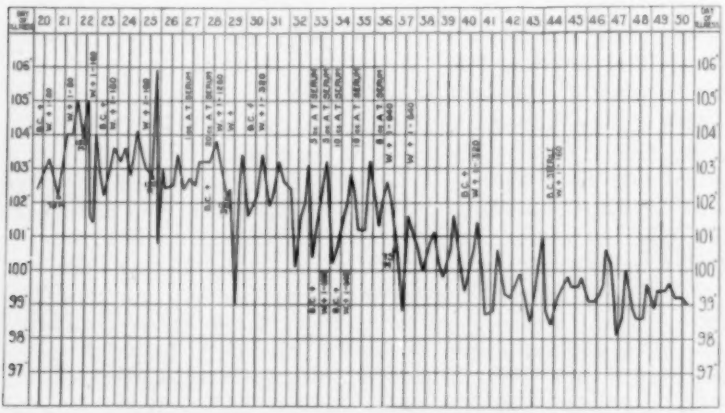

Fig. 1.—Temperature curve and other data in Case 47. The data in these charts are abbreviated as follows: B. C.=Blood culture; W.=Widal; A. T. Serum=Antityphoid serum; 75 M=Sensitized vaccine corresponding to 75 million typhoid bacilli.

Group 2. Benefited Cases.—Of these cases there were thirteen, or 24.5 per cent. in our series. In these cases one or more doses of vaccine led not only to temporary amelioration but to a lytic type of defervescence, the successive drops in temperature being related directly to the vaccine treatments. In cases of this group the duration of the disease was apparently shortened and the course markedly ameliorated. (Fig. 2, Case 43).

Group 3. Aborted Cases.—Cases totaling twenty-two, or 41.5 per cent., of our series. In these cases the injection of vaccine led to a more or less critical fall of temperature directly associated with the vaccine injection. (Fig. 3, Case 35).

TABLE 2.—Relatively Unaffected Cases

No.	Age	Widal	Blood Culture	Treatment, Day	Number of Treatments	Permanent Normal Temp.,* Day	Remarks
2	14	0	0	14	7	Died 32d day	Severe case complicated by laryngeal symptoms. Necropsy refused.
6	20	+80	+	6	4	38	Severe case. White count rose from 8,000 to 21,000 after vaccine treatment.
11	7	+	+	5	5	28	Severe case. Delirium. Abdominal pain.
12	5	+	+	7	4	29	Severe case. Delirium. Abdominal pain.
13	25	+	+	15	3	30	Severe case. Temperature distinctly lower following vaccine treatment, but course of disease not shortened.
14	20	+50	+	15	5	48	Severe case. No effect on temperature course.
16	45	+	+	12	5	49	Severe case. No effect on temperature course.
18	30	+320	+	15	4	45	Severe case. Temporary effect on temperature course.
20	35	0	+	7	2	Died 10th day	Severe. Very toxic. Patient unconscious when first seen.
44	22	+40	+	14	4	52	Moderately severe. Slight symptomatic improvement following injection of vaccine.
47	62	+80	+	21	6	48	Severe case. Blood culture positive to fortieth day of disease.
48	14	0	+	21	1	Died 26th day	Severe case. Toxic. Organism from blood at first inagglutinable. Exsanguinating hemorrhage.
57	56	+1,280†	+	21	1	Died 27th day	Severe case. Temperature normal after vaccine treatment. Died six days later from hemorrhage and perforation.
61	19	+320	+	14	5	43	Severe case. Muscle spasm. One colony B. typhosus per c.c. blood on twenty-second day. Blood sterile three days later following combined vaccine and serum treatment.
66	55	+40	+	14	4	Died 30th day	Severe case. Muscle spasm. Blood culture positive on twenty-fifth day of disease. Three hours after hemorrhage and perforation took place, patient was operated on and perforation closed. Patient's general condition was so serious that death followed in spite of there being practically no gross soiling of the peritoneal cavity.
72	20	+160	+	11	4	57	Severe case; three colonies B. typhosus in 1 c.c. blood on eleventh day. Blood sterile ten days later, following serum and vaccine treatment. Relapse began on thirty-first day, lasting until fifty-seventh day.
75	40	+80	+	15	5	35	Severe case; one colony B. typhosus in 2 c.c. blood on nineteenth day. Positive with 1 c.c. blood until twenty-eighth day. Blood sterile on thirty-first day and defervescence occurred four days later. Hyperleukocytic crisis 17,000 to 18,000 after vaccine treatment.
79	19	+320	+	5	4	29	Severe case. Toxic and comatose. Symptomatically improved by treatment but course not notably shortened.
	28	114	98%	13	4	41	

* A temperature below 100 F. by rectum for entire day.

† This case with high Widal is not an exception. The first injection of vaccine brought the patient's temperature down about three degrees during three days. He died three days later of perforation. This Widal titer is therefore not included in the average.

TABLE 3.—Benefited Cases

No.	Age	Widal	Blood Culture	Treatment, Day	Number of Treatments	Permanent Normal Temp., Day	Duration of Treatment, Days	Remarks
23	21	+160	+	6	2	21	15	Laboratory infection. Blood culture positive fourth day. Symptomatically well on thirteenth day. Afternoon rise in temperature for several days thereafter.
32	10	+1,280	+	11	3	32	21	Severe case. Very toxic. Treatment very cautious because of slight intestinal hemorrhage.
38	18	+40	+	9	3	27	18	Severe case. Toxic, with muscular spasticity. Blood culture positive after three vaccine treatments.
39	8	+160	+	11	5	30	19	Severe case. Complicated by pyelitis. W. B. C. rose to from 10,300 to 25,000 after each vaccine treatment.
42	23	+160	+	13	4	26	13	Moderately severe. Marked diarrhea. W. B. C. rose from 7,800 to 16,250 after vaccine treatment.
42	25	+80	0	16	2	26	10	Mild case.
46	24	+40	+	11	3	28	17	Severe case. Blood culture sterile three days after combined vaccine and serum treatment.
49	28	+40	+	15	3	34	19	Severe case. Blood culture sterile 72 hours after first vaccine treatment. Symptomatically well twenty-third day. Leukocytes rose from 7,400 to 15,200 after treatment.
50	7	+40	+	16	1	28	12	Severe case. Toxic and distended.
74	26	+160	+	18	3	30	12	Moderately severe. Blood culture positive twenty-second day. Hyperleukocytic crisis of 15,200 after vaccine treatment.
81	35	+80	0	11	3	21	10	Mild case.
94	30	+80	+	7	3	23	16	Moderately severe. Blood culture sterile four days after second vaccine treatment.
104	23	+50	+	18	2	28	10	Moderately severe.
	21	+182	84.6%	12.4	2.8	27	14.7	

TABLE 4.—Abortively Recovered Cases

No.	Age	Widal	Blood Culture	Treatment, Day	Number of Treatments	Permanent Normal Temp., Day	Duration of Treatment, Days	Remarks
1	70	+	....	12	1	18	6	Temperature had ranged from 100 to 103 for ten days previous to vaccine treatment.
5	30	+1-80	0	5	3	21	16	Severe case. Hyperleukocytosis of 20,300 following injection of vaccine.
10	14	+	....	10	1	16	6	Moderately severe case.
21	53	+640	0	15	2	21	6	Mild.
26	14	+	....	16	2	23	7	Mild.
27	38	+160	+	10	3	19	9	Blood cultures sterile 24 and 96 hours after vaccine treatment. W. B. C. 4,000 to 14,000.
31	19	+320	0	10	1	15	5	Mild.
33	15	+320	0	15	2	24	9	Moderately severe.
34	6	+320	0	14	1	18	4	Mild.
35	28	+160	+	7	2	13	6	Blood cultures sterile 24 and 48 hours after vaccine treatment.
40	20	+640	0	14	2	22	8	Mild. Accompanied by profuse perspiration.
41	8	+160	0	18	2	26	8	Mild.
51	5	+20	0	6	1	8	2	Mild. Two of three children ill at same time. Blood culture positive in one case.
52	4	+10	0	6	1	11	5	Mild. Two of three children ill at same time. Blood culture positive in one case.
59	5	+20	+	10	2	16	6	Moderately severe. Blood culture sterile four days after first treatment.
62	38	+160	0	17	2	23	6	Moderately severe.
70	21	+160	+	8	3	18	10	Delirious, severe headache. Blood culture sterile 48 hours after first treatment.
73	30	+1,280	+	32	3	40	8	Comatose and involuntary. Pulse 130. Condition critical.
80	14	+160	0	26	1	35	9	Moderately severe.
90	8	+40	+	10	2	23	13	Blood culture sterile five days after first treatment. None taken between.
95	25	+	+	12	2	21	9	Moderately severe.
103*	30	+1-40	+	8	3	11	3	Severe.
	22.5	260	42%	12.7	2—	20	7.3	

* Case of paratyphoid "B".

A consideration of our cases as arranged in these three groups brings out a number of interesting correlations. (Tables 2, 3 and 4).

We see, for instance, that the treatment was begun on the average on about the same day in all three categories (12 to 13) and that the average age of the patients was nearly the same. The first group of "Relatively Unaffected Cases" differs distinctly from the "Abortively Recovered" ones in that the blood cultures were positive in over twice as many cases in the first (95 per cent. as compared with 42 per cent.) and again in the fact that the Widal was much lower on the average in the unaffected cases than in the aborted cases (1 to 114 and 1 to 260).

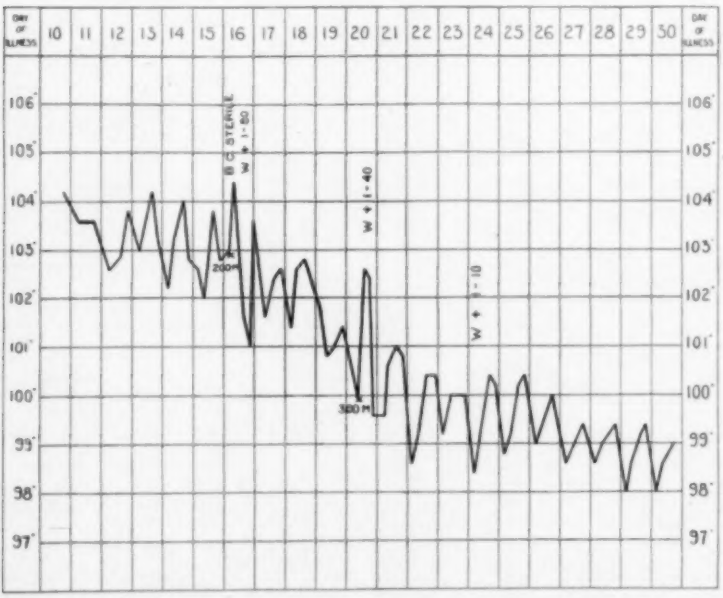

Fig. 2.—Temperature curve and other data in Case 43

The benefited cases (Group 2) lie intermediate between the two, having nearly as high a percentage of blood cultures as Group 1 and nearly as high a Widal average as Group 3.

There is little doubt that the number of typhoid bacilli in the circulating blood bears a distinct relation to the severity of the course of the disease, and the persistence of positive blood cultures indicates an unfavorable outcome (see particularly Schottmüller,[50] Jochmann[51]).

50. Schöttmüller, H.: Die Typhosen Erkrankungen, Handb. der inn. Med., 1912, I, Mohr and Staehelin.

51. Jochmann: Lehrbuch der Infektionskrankheiten, Springer, Berlin, 1914.

Conversely the disappearance of the bacteriemia early in the disease is a favorable indication. From such facts we should expect to find the third group of cases in a general way milder than Group 1.

Whereas it is impossible to judge what the outcome of any one of our cases would have been without treatment, certain impressions of the severity of the cases *before treatment* in relation to the outcome may be of interest (Table 5).

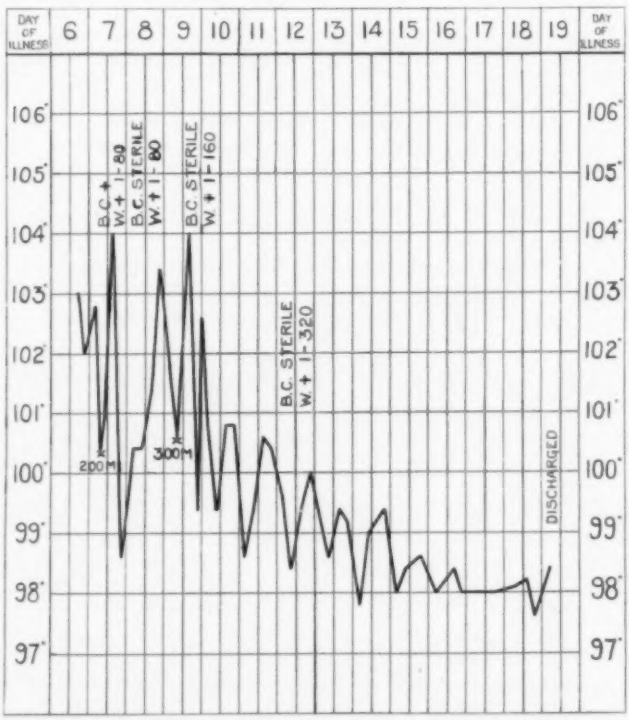

Fig. 3.—Temperature curve and other data in Case 35.

TABLE 5.—General Condition of the Cases Before Treatment

	Severe	Moderate	Mild
Group 1, unaffected	15	3	0
Group 2, benefited	6	5	2
Group 3, aborted	4	7	11

It is evident that the milder cases were more likely to be favorably affected by treatment than the severe ones. In this connection it may be wise to forestall criticisms that might be made to our interpretation of the abrupt recoveries as due to the injection of vaccine. It may be suggested by clinicians with an extensive experience in typhoid fever

that they have seen cases (usually a case) in which the temperature fell by crisis in typhoid fever without treatment. We have been able to find little detailed account of such a course in the literature. McRae[52] in his carefully analyzed series of 1,500 cases noted a critical fall in two cases only (0.1 per cent.). He further notes a mild form of typhoid in 44 cases (3 per cent.). These figures added or separately, may be compared with our 41.5 per cent. of rapid recoveries. Of further significance is the direct relation of the vaccine injection to the result

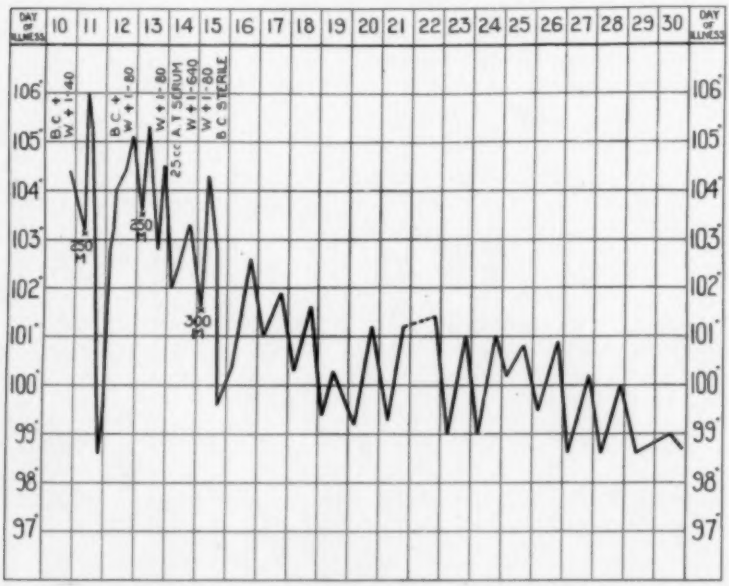

Fig. 4.—Temperature curve and other data in Case 46.

produced and the fact that the average duration of the specific treatment until a *permanent* normal temperature was reached was a little over seven days.

In this connection it may be of interest to contrast the average duration of the disease in the three categories as compared with McCrae's figures (Table 6).

TABLE 6.—DURATION OF DISEASE AS CONTRASTED WITH MCCRAE'S FIGURES

Group 1, unaffected	41	days
Group 2, benefited	27	days
Group 3, aborted	20	days
Real average	27.6	days
Average of McCrae's 1,500 cases	31	days

52. McCrae: Osler's System of Medicine, Ed. 1, ii, 70.

The relative intensity of the Widal reaction in the favorably affected cases as compared with the others seems to us of peculiar significance. It may be taken as an indication of the degree of successful response that the patient has made to the infection. We do not wish to assume that the agglutinin titer is any true measure of resistance, but it often runs parallel to those antibodies that are responsible for tipping the balance in the patient's favor. Our conception of the recovery that is favored by the intravenous injection of sensitized vaccine would be that it is due first to a hyperleukocytosis produced in maximum degree by the use of tropinized bacteria, and secondly to the action of the patient's own antibodies on the circulating bacteria. Such a conception to be proved would necessitate an estimation of the patient's tropins in relation to results produced rather than the agglutinins. The establishment of an accurate method for such determinations is at present engaging our attention.

A further corollary of this hypothesis should show that the bacteria in the patient's blood disappear or diminish in the presence of a hyperleukocytic rise and the simultaneous presence of suitable antibodies. This was found to be the case in rabbits in the experiments of Gay and Claypole. Although it has not been possible to take an extensive series of blood cultures in many of our cases, our results show that in ten cases positive blood cultures became sterile in from one to three days after injection of vaccine. We contemplate a more extensive series in this direction. In addition to diminishing the bacteremia or actually sterilizing the patients, as would seem to be the case in many of our abortively recovered cases, the injection of sensitized vaccine in the majority of cases is accompanied by an increase of the Widal titer which, as we have seen, is of favorable prognostic significance.

In view of the probable mechanism of recovery in typhoid fever induced by the intravenous injection of sensitized vaccine, it might seem reasonable in those cases associated with a low Widal titer in which less favorable results may be anticipated, to supply the necessary antibodies artificially. This could be done by the use of an immune serum. One is not impressed with the results hitherto attained in the serum therapy of typhoid (Chantemesse,[53] Rodet,[54] Rodet and Lagriffoul,[55]

53. Chantemesse, M.: Toxine typhoide soluble et serum antitoxique de la fiévre Typhoide, Prog. méd., 1898, Series 3, vii, 245.

54. Rodet, A.: Die Serotherapie beim Typhus, Handb. der serum Therapie, A. Wolf-Eisner, München, 1910.

55. Rodet, A., and Lagriffoul: La Serotherapie de la Fiévre Typhoide, Presse méd., 1910, xviii, 969.

Lüdke,[56] Andriescu and Ciuca,[57] and Koenigsfeld[58]). We have no certainty whether such a serum should be antiendotoxic, or according to our own working hypothesis, largely tropic. While waiting for further experimental evidence on this point we have felt justified in certain of our cases, particularly in those with a low Widal, in giving intravenous injections of the serum of goats that had received repeated subcutaneous and intravenous injections of several strains of living *B. typhosus*. Whereas the result with such serums has not been in many instances striking, it has at all events shown the harmlessness of goat serum even when given in fairly large quantities intravenously.

Fresh goat serum was first shown to have no hemolytic or hemagglutinative effect on human blood corpuscles. In each case where intravenous injection was intended 1 c.c. of goat serum (either normal or immune) was given subcutaneously twenty-four hours previously to avoid possible anaphylactic shock. In all eight cases were given intravenous injections ranging from 20 to 95 c.c. In no instance was any untoward immediate symptom noted, and in only one case was a slight urticaria found subsequently. This latter finding may be contrasted with the usual results which follow the intravenous injection of large amounts of immune serums from the horse (Cole[59]). In all instances, as expected, the infusion of a relatively considerable amount of antiserum caused an immediate rise of the agglutinins in the patient and in several instances the serum injection alone seemed followed by a symptomatic improvement and slight defervescence.

In one case (Fig. 4, Case 46) which we trust will not prove to be exceptional, two injections of vaccine in a patient with low Widal and positive culture early in the disease (tenth day) were followed by persistent positive cultures and only temporary temperature fall. The administration of 25 c.c. of antityphoid serum intravenously followed the next day by a third dose of vaccine, led to sterilization of the blood stream and immediate defervescence. In this case at least it seems evident that the cure was due to the mixed serum and vaccine treatment.

56. Ludke, H.: Die Serumtherapie des Abdominal typhus, München. med. Wchnschr., 1912, xxv, 907; Die Behandlung des Abdominal typhus mit Intravenosen Injektionen von Albumosen, München. med. Wchnschr., 1915, xxviii, 321.

57. Andriescu, C., and Ciuca, M.: De l'action du Serum antityphique de Besredka sur l'evolution de la fiévre Typhoide, Ann. de l'Inst. Pasteur, 1913, xxvii, 170.

58. Koenigsfeld, H.: Ein neues Prinzip der Serumtherapie bei Infektionskrankheiten mit besonderer Berucksichtigung des Typhus abdominalis, München. med. Wchnschr., 1915, lxii, 253.

59. Cole, R.: Pneumococcus Infection and Lobar Pneumonia, THE ARCHIVES INT. MED., 1914, xiv, 56.

FATAL CASES

It will be seen from Table 2, comprising our relatively unaffected cases, that there have been five fatal cases in our treated series, or a little over 9 per cent., which does not differ from McCrae's large series with the same mortality. Of course no mortality average is of great significance in so small a series as our own. It is to be noted that a disproportionate number of our cases have died from the "accidents" of typhoid, if we may so call them. There was hemorrhage in three cases, two of which were followed by perforation. These two complications should account for death in 40 per cent. of the cases according to McCrae. In our own series they represent 60 per cent. of the fatalities. It should be noted that in two of these cases the treatment was not begun until the twenty-first day. True typhoid toxemia was the cause of death in only one of our cases (20). Case 2 was toxic, but probably died of some laryngeal complication; necropsy was refused.

RELAPSES

There have been five relapses in our series (9 per cent.), somewhat lower than McCrae's average of 11.4 per cent. It should be noted that two relapses have occurred in our cases listed as abortively recovered after discharge from the hospital; both promptly responded to a single reinjection of vaccine. The early discharge of the patient we believe had a distinct influence on the occurrence of these relapses. During the latter part of our series we have followed the intravenous treatments with a series of three subcutaneous injections of the vaccine in the dosage ordinarily employed for prophylaxis (1/10 mg.=750 million). In twelve cases in which this treatment has been fully carried out there have been no relapses, whereas the five relapses occurred in forty-one cases in which no such treatment was employed or completed. These subsequent subcutaneous injections may be followed by slight rise of temperature.

SPECIFICITY OF TREATMENT EMPLOYED

In view of the relative success that has been reported by means of certain nonspecific methods of treating typhoid fever, it may be well to express our conception of the relation of our method of treatment to such methods.

Early in the history of the treatment of typhoid by vaccines Rumpf[2] questioned the specificity of the results claimed by Fraenkel[1] on the ground that he had obtained similar favorable effects by the use of preparations of *B. pyocyaneus*. That such results were at least relatively less effective would seem to be shown from the subsequent work of Kraus and Buswell[3] and Presser.[4] Kraus[60] has obtained abortive

60. Kraus, R.: Ueber Bakteriotherapie akuter Infektionskrankheiten, Wien. klin. Wchnschr., 1915, xxviii, 29.

cures in typhoid by the use of colon vaccines. Ichikawa has cured paratyphoid, as we did, by sensitized typhoid vaccine. Lüdke[55] has mentioned favorable results by the simple use of a deutero-albumose. Letulle and Mage[61] and Gay[62] have treated cases of typhoid by means of a preparation of colloidal gold (colibiase). We believe such favorable results, which we are quite ready to accept, are in reality in perfect harmony with each other and with our own choice of treatment. All of these substances including colloidal gold[63] produce hyperleukocytosis, particularly when injected into the circulation. Leukocytic extracts probably act in the same way in those cases in which they are undoubtedly effective. Any of these substances could therefore be expected to cure a percentage of cases of typhoid owing to the dual mechanism of a hyperleukocytosis plus antibodies in the patient.

Intravenous treatment by means of sensitized typhoid bacilli differs from the above methods in two ways:

1. Owing to the fact that the injected protein is sensitized or tropinized, the response on the part of the leukocytes is much more intense and effective (Gay and Claypole[12]).

2. Typhoid vaccine aids in building up the active immunity of the patient against typhoid fever as no other preparation can.

SUMMARY

This article deals with the study of 105 cases of suspected typhoid fever in which we were allowed to examine the patients through the great courtesy of a number of physicians in Alameda and San Francisco Counties. Thorough laboratory examinations in most of these cases by blood cultures and Widal tests and in a number the search for the typhoid bacilli in the stools and urine, offer certain facts of interest in the differential diagnosis of typhoid fever. In 65 of the 105 cases the diagnosis of typhoid fever was made from both clinical and laboratory data. In these 65 cases the Widal was positive in 60 (93.7 per cent.) and as early as the fifth day, the high percentage of results being due, in a large measure, to the method employed, namely, the use of the macroscopic method and a formaldehydized culture of the typhoid bacillus. Of the blood cultures taken in 58 cases there were 40 positive (70 per cent.), including a case first seen on the thirty-second day. In only one case of the 65 were both Widal and

61. Lutelle, M., and Mage: Traitément la Fiévre Typhoide par l'or colloidal en injections intraveineuses, Bull. de l'Acad. méd., Paris, 1914, lxxii, 421.

62. Gay: Un Traitément pratique de la fiévre typhoide aux armees, Presse méd., March 4, 1915, p. 67.

63. See discussion by Robin and Chantemesse in loc. cit., Ref. 60.

blood culture negative, which case was diagnosed by the presence of *B. typhosus* in the stools.

Of the 40 cases excluded as not being typhoid fever on a laboratory basis, 36 could ultimately be excluded on both clinical and further laboratory examination. There remain 4 cases which on clinical evidence alone may have been typhoid, but which are not included in our series owing to the fact that laboratory proof was lacking. It may incidentally be remarked that 2 of these cases were treated by the method described, with abrupt recovery. Of the 65 cases it was impossible for various reasons that are stated, to treat 12. The remainder of the cases, however, were all treated without choice.

There remain, then, a series of fifty-three cases of typhoid fever in which the diagnosis was absolutely certain both on clinical and laboratory grounds. We have not attempted to influence the ordinary symptomatic treatment of these cases, which differs as much as might be expected in a group of over fifty physicians. The patients, moreover, were cared for under varying conditions in private homes and hospitals, some of them even without the attention of a trained nurse. This variation in care and location of the cases had undoubtedly an effect on the mortality and has made thorough laboratory examinations, such as leukocyte counts at frequent intervals, impossible in all the cases.

The mortality in these cases has been precisely what one would expect under the best hospital conditions (McCrae), namely 9 per cent., which we regard as suggesting that, under uniform conditions, with our method of treatment the mortality would have been less than the average. The mortality has been composed, to a large extent, of what may be termed the "accidents" of typhoid, namely 60 per cent. of the deaths by hemorrhage or perforation.

Our method of treatment has consisted in the intravenous injection of 1/50 to 1/25 milligram (150 to 300 million bacteria) of a sensitized, polyvalent, killed typhoid vaccine sediment prepared after the method of Gay and Claypole. This injection gives rise to a series of symptoms characterized particularly by a chill, rise and fall of temperature and leukopenia followed by hyperleukocytosis. The fall of temperature with its attendant hyperleukocytosis leaves the patient at least temporarily benefited, and the benefit and normal temperature may be permanent. Thus in 66 per cent. of the cases a distinct benefit was obtained, as shown by lowered temperature, disappearance or amelioration of subjective symptoms and an apparently accelerated recovery. In 41.5 per cent. of this 66 per cent. the recovery was of an abortive form with a critical fall of temperature and a permanent normal temperature established within a few days. This permanent normal tem-

perature was reached on an average seven days after beginning treatment in these cases. There remains, however, 34 per cent. of cases which are classified as relatively unaffected. We regard this classification as underestimating the beneficial results for reasons given. In none of the cases did the use of the vaccine have any apparent harmful effect on the case, although in four, in which too large a dose was used, the symptoms were somewhat alarming.

A series of subcutaneous injections following the intravenous treatment apparently aids in preventing relapses.

We regard the mechanism of benefit and cure in these cases which were affected by the treatment as due to a combination of specific hyperleukocytosis and the presence of antibodies (tropins?) in the patient's blood. The injection of vaccine could be shown in a number of cases to be followed by the disappearance or diminution of bacteremia and usually also by an increase in the Widal. In those cases which did best the Widal was originally high and those cases which showed the least effect had the low Widals. The cases judged as "mild" before treatment began did better on the whole than those regarded as "severe." There were, however, a number of severe cases which showed abrupt recovery or benefit.

On the hypothesis that successful results are due to the strength of the antibodies already established in the patient, we have ventured in severe cases with low antibody content, to combine with the vaccine treatment the intravenous injection of considerable amounts of typhoid immune serum from goats. These cases, although few in number, suggest that this type of treatment with further elaboration might increase the percentage of favorable results.

We regard the use of sensitized vaccine as being better for intravenous injection than plain typhoid vaccine or less specific methods of treatment that have been suggested by other authors, owing to the fact tnat sensitized typhoid vaccine produces a specific form of hyperleukocytosis of maximum degree (Gay and Claypole), and may also be shown to be followed by an increase in active immunity of the patient against the disease.

THE INTERPRETATION OF A POSITIVE NITROGEN BALANCE IN NEPHRITIS*

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It has been the practice of clinicians for many years to treat cases of nephritis by means of a dietetic therapy, in which the various forms of nitrogen-containing food have beeen much restricted. One of the several reasons for curtailment of protein in the dietary has been that a damaged kidney cannot readily excrete the end products of protein metabolism, as measured by the nitrogen, and that a distinctly harmful result follows such a retention. The determination of a nitrogen balance in nephritis has been considered one of the valuable tests of the ability of the kidney to eliminate this substance. In the interpretation of this procedure, three factors have been considered essential:

1. An accurate determination of the food nitrogen.

2. An accurate determination of the output of the nitrogen in the urine and feces.

3. A constant intermediary nitrogenous metabolism. The first two of these demands are readily fulfilled, the third is vague in its requirements and no definite standards have been established for it in clinical medicine. Studies in nephritis are largely based on the criteria set by Ascoli and von Noorden. These authors acknowledge that there may be either an increased protein catabolism or an assimilation of ingested nitrogen within the body which may cause an apparent loss or gain of nitrogen to the organism for which the kidney is in no wise responsible. The problem which has to be solved is, how are such possible changes to be eliminated? Ascoli and von Noorden[1] in their own words make the following suggestions in this connection:

Ascoli: These observations are based on the experience that an individual who is receiving a medium or at least a sufficient amount of food with not too

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* From the Medical Clinic of the Johns Hopkins Hospital.

1. Ascoli: Wir gehen bei diesen Beobachtungen von der Erfahrung aus, dass der Mensch bei einer mittleren oder eben genügender Nahrungszufuhr mit nicht allzu geringem Eiweissgehalte innerhalb 24-stündiger, noch besser aber mehrtägiger Beobachtungszeiten ziemlich genau so viel Stickstoff—in Form der verschiedenartigsten Schlacken—in Harn und Kot ausscheidet, als mit der Nahrung, vor allem mit dem Eiweiss, eingeführt wird. Bleibt unter diesen Umständen die Ausfuhr gegen die Einfuhr merklich zurück, so kann es sich zwar bei heruntergekommenen Personen um Fleischansatz aus dem eingeführten Eiweiss handeln; meist kann aber ziemlich unbedenklich angenommen werden,

low a protein content, will excrete in the urine and feces in a twenty-four-hour, or better still, several-day period of observation, an amount of nitrogen in the form of the various waste products approximately equivalent to that taken in. If the amount excreted is markedly less than the quantity ingested, it is possible that some of the retained material is utilized to build up the tissues, in the case of individuals who are emaciated; but, as a rule, it may be assumed without question that particular nitrogenous residues which should be excreted are being retained in the blood, in the organs, and by chance, in edematous accumulations as waste nitrogen.

Von Noorden: It is evident that statements of nitrogen elimination in the urine are of value only when the amount ingested, as well as the quantity excreted in the feces, is considered and if the observations are sufficiently prolonged. The diet must be at least fairly constant and the nitrogen content must be easily ascertained. If the observations do not attain these standards, then they are deserving of consideration only when extreme deviations from the normal occur, as in the occasional cases cited by Frerichs, Bartels and S. Rosenstein, in their publications.

These statements are not precise and allow of much latitude in their interpretation. To give them more concrete form we may compare them with the actual fulfillment in a given case. Von Noorden claims, in the above quotation, that in order to set aside changes in the intermediary metabolism, the diet must be fairly constant, the observation must be carried on over a considerable period and that if these conditions are not fulfilled, then the changes noted must be very striking in order to be of value. In a patient,[2] a boy of 11 years, whose data were considered extremely important as being the first case in which protein destruction was demonstrated in uremia, the period of observation was ten days, the nitrogen content of the food was 7.8 gm. on every day excepting two, when no food whatsoever was taken, and the result of the nitrogen balance for the whole period was a loss of 20.8 gm., or a little over 2 gm. of nitrogen per day. This may be considered a very lax interpretation of the criteria set for such experiments. They are, however, characteristic of the manner in which they have been adhered to by many authors who have reported results with nitrogen balances in clinical work. The cases which are reported here all fall within the limits set by these figures and show how thoroughly inadequate they are in rendering the course of intermediary nitrogenous metabolism a negligible factor.

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dass eine Anstauung von zur Ausscheidung bestimmten Schlacken im Blute, in Organen, allenfalls in sich ansammelnden Oedemenstatthat. Vorlesungen über Urämie, Jena, 1903, p. 166.

Von Noorden: Es ist klar, dass die Angaben über N-Elimination nur Wert haben, wenn sie die Bestimmung der Nahrungszufuhr und des Kotstickstoffs einschliessen und die Versuche nicht allzu kurz sind. Zum mindesten muss die Diät einigermassen konstant under ihr N-Gehalt leicht zu berechnen gewesen sein. Erfüllen die Versuche diese Bedingungen nicht, so verdienen sie nur Beachtung, wenn geradezu extreme Abweichungen vom Gesundhaften vorlagen, wie in einzelnen Fällen, die Frerichs, Bartels, S. Rosenstein in ihren Handbüchern zitieren. Von Noorden: Handbuch der Pathologie des Stoffwechsels, Berlin, 1906, i, 978.

2. Von Noorden: l. c. in Note 1, p. 972.

The method of Folin and Denis[3] for the total nonprotein nitrogen of the blood, which was the one used in the present series, opens the way to a deeper knowledge of nitrogen metabolism. With three data, the nitrogen intake, the nitrogen output, and the amount of waste nitrogen in the blood, at our command, the trend of intermediary protein metabolism, as well as the power of the kidney to eliminate nitrogen, become apparent. It has previously been shown that, in experimental uranium nephritis, at least, an increased protein catabolism takes place and may be detected by these means.[4] The present observations deal with the second phase of this subject, namely, the influence that the assimilation of ingested nitrogen may have on the interpretation of the nitrogen balance.

The patients investigated in this connection were all cases of chronic interstitial nephritis of moderate severity. The first four cases studied were at the outset put on a "low protein" diet and subsequently on a "high protein" diet. These diets were as follows:

LOW PROTEIN DIET

Breakfast: Sherry, 30 c.c. Baked apple, stewed prunes, orange. "Hominy cornstarch cereal.[5] Cream, 15 c.c.

Dinner: Sherry, 30 c.c. Potato, baked or mashed. String-beans, cabbage, carrots, lettuce, onions, tomatoes, cucumber pickles. Fruit cornstarch pudding, fruit tapioca pudding.

Supper: Same as dinner.

Salt, sugar and butter may be used as desired and need not be weighed. All other food eaten must be weighed or measured.

HIGH PROTEIN DIET

Bread, coffee, cream, milk, cocoa made with milk, eggs, oatmeal, broth, baked or mashed potato, roast beef, beefsteak, chopped beef, chicken, custard, rice pudding.

Salt, sugar and butter may be used as desired and need not be weighed. Whisky and sherry may be used to make up eggnogs, etc., and need not be measured. All other food eaten must be weighed or measured. Measure the milk and cream separately from the coffee, oatmeal, etc., when serving them.

Make the diet as full as possible, allowing much meat and broth.

Each patient was given sufficient food from these lists to satisfy the appetite. The amounts to be consumed were weighed; duplicate determinations for nitrogen were made on every sample of food. The average nitrogen values thus obtained were as shown in Table 1.

These figures vary somewhat from those given in the standard food tables. They also do not agree with analyses made on the food from the same hospital kitchen a few months previously. It would seem that for very accurate work samples of the actual food consumed must

3. Folin and Denis: Jour. Biol. Chem., 1912, xi, 527.
4. Mosenthal: THE ARCHIVES INT. MED., 1914, xiv, 844.
5. Two-thirds hominy, one-third cornstarch.

necessarily be analyzed and that previous analyses or dietary tables cannot be relied on absolutely. It will be seen from the protocols that the nitrogen intake on the days of low protein diet was between 1.54 and 4.30 gm., while on the high protein diet it varied from 16.83 to 47.37 gm. The intake in each individual, however, was much more constant on the days of similar diet than these maximum variations for all the cases indicate.

TABLE 1.—NITROGEN CONTENT OF THE FOODS USED IN THE HIGH AND LOW PROTEIN DIETS

Article of Food	Percentage of Nitrogen
Bread	1.47
Coffee	0.03
Cream	0.41
Milk	0.53
CEREALS	
*"Hominy cornstarch"	0.13
Oatmeal	0.36
FRUITS	
Baked apple	0.04
Orange	0.16
Stewed prunes	0.14
VEGETABLES	
Cabbage	0.16
Carrots	0.10
Lettuce	0.24
Onions	0.17
Cucumber pickle	0.10
Baked potato	0.48
Mashed potato	0.40
Stringbeans	0.23
Tomatoes	0.23
DESSERTS	
Blackberry cornstarch pudding	0.05
Prune cornstarch pudding	0.07
Custard	0.92
Soft custard	0.81
Apple tapioca pudding	0.02
Peach tapioca pudding	0.06
Rice pudding	0.72
MEAT PRODUCTS	
Broth	4.00
†Chopped beef	3.36
Cold roast beef	5.40
†Beefsteak	3.90
Chicken	5.67
‡Eggs	2.13

*Two-thirds hominy, one-third cornstarch.

†These nitrogen determinations were made on uncooked food; all the other determinations on prepared foods.

‡Eggs are the only article of food listed the nitrogen value of which was not actually determined. The figures were taken from standard food tables of Atwater and Bryant (Bulletin 28, revised edition, U. S. Department of Agriculture).

METHODS

The Kjeldahl method was used for the quantitative determinations of nitrogen in the food, urine and stools. In the first four cases, the stools were collected in composite periods, corresponding to the duration of high and low protein diets. They were marked off by the alternate ingestion of charcoal and carmin. The fecal material was dried in evaporating dishes, after the addition of a small amount of sulphuric acid, ground in a mortar and thoroughly mixed before being analyzed. In the last two cases, the stools were collected in twenty-four-hour periods, without demarcation, and aliquot portions of the thoroughly mixed moist feces were used for analysis. The procedure of Folin and Denis[6] was employed to obtain the figures for nonprotein nitrogen of the blood. The original method was adhered to, except that a combined process of distillation and aeration was substituted for aeration alone.

TABLE 2.—DATA OF CASE 1.

Diet	Weight, Lbs.	Nitrogen, Gm. in 24 Hours					Nonprotein Nitrogen of the Blood, Mg. per 100 c.c.
		Urine	Feces	Total Output	Intake	Balance	
Low protein.....	147¼	6.00	.75	6.75	3.33	— 3.42	31.8
Low protein.....	146½	4.80	.75	5.25	3.13	— 2.12	
Low protein.....	145½	3.36	.75	4.11	3.17	— 0.94	
Low protein.....	146½	3.15	.75	3.90	3.37	— 0.53	
High protein.....	147	6.23	.82	7.05	26.46	+19.41	29.7
High protein.....	147½	6.16	.82	6.98	31.25	+24.27	
High protein.....	148	11.38	.82	12.20	32.20	+20.00	
High protein.....	147	5.59	.82	6.41	21.68	+15.27	
High protein.....	146½	10.20	.82	11.02	24.03	+13.01	37.0

In each case there was a slight loss of nitrogen on the low protein diet. On the first day of this diet the deficit was rather large in one instance. This was, in all probability, due to the elimination of nitrogenous waste products which had previously been retained. On the high protein diet there was very marked nitrogen retention in all the patients. Thus, considering the whole period of observation, the nitrogen balance is distinctly positive. (See Table 6.)

In spite of this, the nonprotein nitrogen of the blood remained at a comparatively low level—33.9 to 38.1 mg. per 100 c.c.—exceeding the normal by only a slight margin. (The upper normal limit of total nonprotein nitrogen of the blood has been variously placed at 26[6], 30[7], 32[8], 37[9] and 40[10] mg. per 100 c.c.).

6. Folin and Denis: Jour. Biol. Chem., 1913, xiv, 29.
7. Frothingham and Smillie: THE ARCHIVES INT. MED., 1915, xv, 204.
8. Tileston and Comfort: THE ARCHIVES INT. MED., 1914, xiv, 620.
9. Folin and Denis: Jour. Biol. Chem., 1914, xvii, 487.
10. Foster: THE ARCHIVES INT. MED., 1915, xv, 356.

How are these results to be interpreted? Under the given conditions, the usual conclusion has been that the kidney is insufficient and that consequently the urinary nitrogen cannot keep pace with the quantity ingested. In such instances it is supposed that the retained nitrogen circulates in the body as nonprotein nitrogen and manifests itself by a rise in its concentration in the blood. It is, therefore,

TABLE 3.—DATA OF CASE 2.

Diet	Weight, Lbs.	Nitrogen, Gm. in 24 Hours					Nonprotein Nitrogen of the Blood, Mg. per 100 c.c.
		Urine	Feces	Total Output	Intake	Balance	
Low protein.....	96¾	6.10	1.27	7.37	1.93	— 5.44	36.0
Low protein.....	97½	2.48	1.27	3.75	2.24	— 1.51	
Low protein.....	98¼	2.89	1.27	4.16	2.34	— 1.82	
Low protein.....	97¾	1.43	1.27	2.70	1.87	— 0.83	
High protein.....	97¼	6.07	1.58	7.65	16.83	+ 9.18	29.7
High protein.....	97	7.75	1.58	9.33	26.71	+17.38	
High protein.....	97	7.36	1.56	8.90	22.54	+13.58	
High protein.....	97½	9.80	1.58	11.38	26.35	+14.97	
High protein.....	97¾	7.68	1.58	9.26	19.13	+ 9.87	38.1

TABLE 4.—DATA OF CASE 3.

Diet	Weight, Lbs.	Nitrogen, Gm. in 24 Hours					Nonprotein Nitrogen of the Blood, Mg. per 100 c.c.
		Urine	Feces	Total Output	Intake	Balance	
Low protein.....	.....	2.73	1.41	4.14	1.82	— 2.32	21.2
Low protein.....	112	2.55	1.41	3.96	1.68	— 2.28	
Low protein.....	111¾	2.41	1.41	3.82	1.98	— 1.84	
Low protein.....	110½	1.77	1.41	3.18	1.54	— 1.64	
High protein.....	111	4.29	0.94	5.23	18.34	+13.11	25.4
High protein.....	.....	7.45	0.94	8.39	17.11	+ 8.72	
High protein.....	112¾	9.05	0.94	9.99	19.22	+ 9.23	
High protein.....	113	10.70	0.94	11.64	20.97	+ 9.33	
High protein.....	111¼	8.19	0.94	9.13	19.73	+10.60	33.9

necessary to ascertain, approximately, at least, to what height the retention of a given amount of nitrogen should raise the figure for waste nitrogen in the blood if this hypothesis is to be supported.

It is known from the work of Marshall and Davis[11] that urea is evenly distributed throughout the body, except in certain tissues, as the

11. Marshall and Davis: Jour. Biol. Chem., 1914, xviii, 53.

fat, bone, cartilage, teeth, outer layers of the skin, etc., which do not take up urea. Since the greater part of the nitrogen of the food—approximately 85 per cent.—is excreted as urea on a moderately high nitrogenous diet, a substantial increase in the nonprotein nitrogen of the blood would be expected if the kidneys did not eliminate this substance sufficiently rapidly to keep pace with its production. It is at the present

TABLE 5.—Data of Case 4.

Diet	Weight, Lbs.	Nitrogen, Gm. in 24 Hours					Nonprotein Nitrogen of the Blood, Mg. per 100 c.c.
		Urine	Feces	Total Output	Intake	Balance	
Low protein.....	136	17.16	0.75	17.91	3.69	—14.22	46.6
Low protein.....	135	8.83	0.75	9.58	2.15	— 7.43	
Low protein.....	135½	5.86	0.75	6.61	2.86	— 3.77	
Low protein.....	137	3.00	0.75	3.78	4.30	+ 0.52	
High protein.....	139	7.10	1.47	8.57	20.52	+11.95	29.7
High protein.....	140	7.31	1.47	8.78	32.76	+23.98	
High protein.....	138½	12.87	1.47	14.34	27.02	+12.68	
High protein.....	139¼	12.56	1.47	14.03	32.68	+18.65	
High protein.....	139¼	11.77	1.47	13.24	47.37	+34.13	37.1

TABLE 6.—Summary of Observations on the Nitrogen Balance and the Nonprotein Nitrogen of the Blood

	Case 1	Case 2	Case 3	Case 4
Nonprotein Nitrogen of the Blood, Mg. per 100 c.c.				
At beginning of observation.....	31.8	36.0	21.2	46.6
At end of low protein period.....	29.7	29.7	25.4	29.7
At end of high protein period.....	37.0	38.1	33.9	37.1
Nitrogen balance in grams for:				
Low protein period.....	— 7.0	— 9.6	— 8.1	— 24.9
High protein period	+92.0	+65.0	+51.0	+101.4
Total period of observation.....	+85.0	+55.4	+42.9	+ 76.5

time not accurately known how the other nitrogenous products destined for urinary excretion are distributed in the body. Many of these are evidently stored in the tissues in much greater concentration than they are found in the blood.[12] However, these materials are probably being held for further use in the body and may be considered in a vastly different light from those which are to be excreted. For the purposes

12. Van Slyke and Meyer: Jour. Biol. Chem., 1912, xii, 399; 1914, xvi, 197; 1914, xvi, 213. Folin and Denis: Jour. Biol. Chem., 1912, xi, 87; 1912, xii, 141.

of determining the theoretical values of the nonprotein nitrogen of the blood in this series of cases, in which it was supposed that the nitrogen output should have equaled the intake, the total quantity of retained nitrogen has been assumed to be equally distributed in the body. From the figures given by Marshall and Davis it is found that in an individual weighing 70 kilos the retention of 30 grams of urea is equivalent to a rise of 40 mg. per 100 c.c. in the urea of the blood. Applying the same principles to the total non-protein nitrogenous products which are supposed to be excreted by the kidneys, it is seen that for every gram of nitrogen retained, the non-protein nitrogen of the blood should be increased 1.33 mg. per 100 c.c. According to these calculations, in the cases presented here, if none of the retained nitrogen were assimilated or stored, and all of it circulated as waste nitrogen, because the kidneys did not excrete it, the figures shown in Table 7 would be obtained.

TABLE 7.—THEORETICAL AND ACTUAL VALUES OF NONPROTEIN NITROGEN OF THE BLOOD RESULTING FROM NITROGEN RETENTION

	N. Gms. Retained During High Protein Period	Theoretical Value of Nonprotein N. of Blood, Mg. per 100 c.c.*	N. Gms. Retained During Whole Period of Observation	Theoretical Value of Nonprotein N. of Blood, Mg. per 100 c.c.†	Actual Value of Nonprotein N. of Blood, Mg. per 100 c.c.
Case 1.....	92.0	152.0	85.0	144.8	37.0
Case 2.....	65.0	116.2	55.4	109.7	38.1
Case 3.....	51.0	93.2	42.9	78.3	33.9
Case 4.....	101.4	164.6	76.5	148.4	37.1

* These figures represent the values obtained for nonprotein nitrogen of the blood at the beginning of the high protein period plus the theoretical increment due to retained nitrogen.
† These figures represent the values obtained for nonprotein nitrogen of the blood at the beginning of the observation plus the theoretical increment due to retained nitrogen.

Even an extremely large error in these theoretical calculations would not invalidate the conclusion that the retained nitrogen was not circulating as waste nitrogen but had been assimilated or stored by the body tissues. In normal individuals a similar storage or assimilation of nitrogen may occur on a high protein or a high calory diet, as was shown by Wolfe,[13] Lüthje,[14] Lüthje and Berger,[15] von Noorden and Krug,[16] and Bornstein.[17] In the absence of carbohydrate food, such an assimilation of nitrogen is difficult to obtain.[18] Frothingham and

13. Wolfe, Quoted by Lusk: Science of Nutrition, 1909, p. 124.
14. Lüthje: Ztschr. f. klin. Med., 1902, xliv, 22.
15. Lüthje and Berger: Deutsch. Arch. f. klin. Med., 1904, lxxxi, 278.
16. Von Noorden and Krug: Arch. f. Anat. u. Physiol., Physiol. Abt., 1893, p. 371.
17. Bornstein: Pfluger's Arch. f. Physiol., 1901, lxxxiii, 540; Berl. klin. Wchnschr., 1898, xxxv, 791.
18. Lusk.: Science of Nutrition, 1909, Chapter vii.

Smillie[19] report instances of nephritis exhibiting the same phenomena as those discussed here.

TABLE 8.—Data of Case 5.

Nitrogen Gm. in 24 Hours					Nonprotein Nitrogen of the Blood Mg. per 100 c.c.
Urine	Feces	Total Output	Intake	Balance	
11.15	1.46	12.61	11.56	— 1.05	29
8.57	1.31	9.88	14.08	+ 4.20	
13.21	0	13.21	13.23	+ 0.02	
12.79	3.28	16.07	15.11	— 0.96	
6.62	1.96	8.58	19.09	+10.51	
7.25	2.75	10.00	19.91	+ 9.91	
11.00	0	11.00	13.93	+ 2.93	
10.46	2.53	12.99	19.89	+ 6.90	
13.56	1.71	15.36	19.12	+ 3.76	
12.26	1.91	14.17	11.99	— 2.18	28
12.65	1.80	14.45	14.50	+ 0.05	
12.76	0.97	13.73	15.15	+ 1.42	
13.80	0	13.80	17.56	+ 3.76	
13.70	2.94	16.64	13.28	— 3.36	
15.48	0	15.48	14.35	— 1.13	
15.60	4.34	19.94	21.96	+ 2.04	
16.46	0	16.46	18.33	+ 1.87	
14.88	4.43	19.31	24.86	+ 5.55	
14.90	4.45	19.35	24.39	+ 5.04	
12.59	2.78	15.37	23.09	+ 7.72	
13.67	2.42	17.09	24.93	+ 7.84	
13.96	0	13.96	21.91	+ 7.95	
14.85	5.46	20.31	16.61	— 3.70	27

The nitrogen balance for the whole period is + 69.1 gm. The theoretical value of the nonprotein nitrogen of the blood, calculating from the first observation of 29 mg. per 100 c.c., should be 119 mg., provided the retained nitrogen were circulating as waste nitrogen; the actual value is 27 mg.

In view of the fact that the above cases were observed over a short time only, and were put on rather a bizarre diet, it is of interest to note what occurs in individuals studied for a longer period, and on what is an ordinary mixed diet. The following two cases (Cases 5 and 6) were given mixed diets containing a fair amount of meat and broth. They ate what food they chose and the nitrogen was calculated from the amount eaten. Foods which might be expected to vary in their nitrogen content, such as broth, potato, meat, etc., were analyzed in isolated

19. Frothingham and Smillie: The Archives Int. Med., 1915, xv, 204.

samples, and the content of nitrogen in the other articles of diet was estimated according to Atwater and Bryant's[20] food tables. The nitrogen balances in these two instances, therefore, do not possess the accuracy of the first four cases. However, the error is probably not great enough to interfere with the conclusion that a positive nitrogen balance may occur in nephritis under ordinary circumstances without an increase in the nonprotein nitrogen of the blood. Case 5 in twenty-three days retained 69.1 gm. of nitrogen; at the termination of this period, the level of the nonprotein nitrogen of the blood was 27 mg. per 100 c.c., whereas, the theoretical value would have been 119 mg., had the retained nitrogen been circulating as waste nitrogen. Case 6 in seventeen days retained 35.3 gm. of nitrogen, which should have given a theoretical value for nonprotein nitrogen of the blood of 117 mg. per 100 c.c., the actual value being 74 mg., practically duplicating the control figures at the beginning of the observation.

TABLE 9.—DATA OF CASE 6.

Nitrogen Gm. in 24 Hours					Nonprotein Nitrogen of the Blood Mg. per 100 c.c.
Urine	Feces	Total Output	Intake	Balance	
9.99	1.68	11.67	13.20	+1.53	71
10.48	0.61	11.09	11.21	+0.12	
12.86	1.45	14.31	13.44	−0.87	
9.89	4.72	14.61	23.03	+8.42	
13.76	1.22	14.98	20.43	+5.45	
21.13	1.74	22.87	21.34	−1.53	
15.81	0.81	16.62	20.83	+4.21	
15.79	1.63	17.42	19.71	+2.29	
20.70	1.32	22.02	21.89	−0.13	
18.57	0.45	19.02	21.64	+2.62	
18.89	1.40	20.29	22.53	+2.24	
16.94	2.60	19.54	23.83	+4.29	
18.00	2.77	20.77	20.32	−0.45	80
18.48	2.09	20.57	25.30	+4.73	
18.85	3.01	21.86	22.78	+0.92	
16.71	2.80	19.51	21.92	+2.41	
17.46	3.67	21.13	20.14	−0.99	74

The nitrogen balance for the whole period is + 35.3 gm. The theoretical value of the nonprotein nitrogen of the blood, calculating from the first observation of 71 mg. per 100 c.c. should be 117 mg., provided the retained nitrogen were circulating as waste nitrogen; the actual value is 74 mg.

20. Atwater and Bryant: Bull. 28, revised Ed. U. S. Dept. Agriculture.

CASE REPORTS

J. W. (Med. No. 34006). Male, aged 24. The urine shows a large amount of albumin, a moderate number of hyaline, granular and epithelial cell casts, and varies in specific gravity from 1.011 to 1.023. The systolic blood pressure is 175; diastolic, 115. The heart, arteries and other organs show no abnormalities. There was some subcutaneous edema on admission which disappeared shortly and remained absent during the tests. The diagnosis is chronic diffuse nephritis.

E. F. (Med. No. 33757). Male, aged 36. Albumin was discovered in the urine seventeen years ago. For five years there has been polyuria and polydipsia; for two years, failing vision. There is moderate thickening of all the peripheral arteries. The cardiac impulse is forcible in the fifth interspace, 12½ cm. to the left of the median line, indicating some hypertrophy of the heart muscle. There is no edema, nor are there other signs of myocardial insufficiency. The systolic blood pressure varies between 210 and 195 mm. of mercury. The eye grounds show marked albuminuric retinitis. The urine is large in amount, constantly low in specific gravity, contains about 1 gm. of albumin per liter, and on microscopic examination yields a few hyaline casts. The phenolsulphonephthalein excretion is 22 per cent. in two hours. The diagnosis is: Advanced degree of chronic diffuse nephritis (secondary contracted kidney).

DISCUSSION

The conception of the retention of nitrogen in nephritis, as understood by the clinician, generally implies two facts: First, that a positive nitrogen balance is usually due to kidney insufficiency; second, that the retained nitrogen is present in the body as waste nitrogen and circulates in the blood, in part, at least, as nonprotein nitrogen. From the cases described above it becomes evident that one or both of these postulates do not hold true in a great number of instances. It is known that in normal individuals on a high protein diet, containing an adequate carbohydrate portion, a large amount of nitrogen may be retained. The same retention may take place in individuals suffering with nephritis and may thus be misleading if used as a measure of the ability of the kidney to eliminate nitrogen. On the other hand, if it is supposed that the nitrogen is retained because of insufficient kidney action, then, in some cases, as those cited above, there must be a compensatory action on the part of the muscles and other cellular tissues, in storing or assimilating such an excess of nitrogen and not allowing it to appear in the blood as waste nitrogen. The solution of this problem involves the whole question of intermediary protein metabolism, and cannot be fully discussed here.

CONCLUSION

A marked positive balance of nitrogen in cases of nephritis on a mixed diet is not necessarily followed by a corresponding increase in the nonprotein nitrogen of the blood. Until these phenomena are elucidated, discretion must be exercised in interpreting a normal figure

for nonprotein nitrogen of the blood as indicating that no nitrogen retention has taken place, and in considering a positive nitrogen balance as an absolute indication of the inability of the kidney to excrete this substance.

The Johns Hopkins Hospital.

BOOK REVIEW

DIABETES MELLITUS. Designed for the Use of Practitioners of Medicine. By Nellis B. Foster, M.D., Assistant Professor of Medicine, Cornell University. Cloth. Price, $3 net. Pp. 243. Philadelphia: J. B. Lippincott Company, 1915.

Dr. Foster has added to the large existing literature of diabetes, not a monograph containing new discoveries, but a valuable compilation of existing knowledge for the general practitioner. This it frankly purports to be, and that there is need for such a book in English admits of little question. The value of such a compilation resides wholly in the critical judgment exercised by the author in selecting from the vast material in the field, in the point of view from which he surveys that material, and the clearness and order of its presentation. The compilation by a man who has done no independent work in a field can have no value because such a man possesses no basis for critical judgment.

Dr. Foster has been a student of diabetes both from the clinical and the biochemical standpoints, and he has succeeded in selecting what is essential and in presenting with about equal emphasis the important theoretical investigations on which our knowledge of diabetes rests and the practical considerations which determine treatment and prognosis. The book is perhaps best as a sane and simple presentation of the diabetic metabolism. Any clinician who familiarizes himself with the contents of its first eight chapters will have an adequate theoretical basis for his practice. The method of treatment outlined is the orthodox one in vogue for many years, very satisfactorily worked out for the general practitioner with diet tables and reasonably detailed directions. The illustrative cases introduced everywhere make excellent object lessons and add greatly to the usefulness of the chapter on treatment, as well as to that on symptomatology. It is unfortunate for Dr. Foster that his book appeared just before the publications of Allen, which have excited so wide an interest in the application of much more rigorous starvation in the treatment of diabetes than has ever previously been attempted. It is well to point out, however, that the exact limitations of the starvation treatment have not yet been clearly determined, and that only a practitioner who has at hand such knowledge as Dr. Foster's book presents can wisely apply this newer plan of management.

Such criticisms as can be made concern matters of detail. There are a number of errors due to poor proof reading, and some departures from good usage in the language. An important omission of at least one entire line occurs at the top of page 49. On page 19, in discussing the concentration of the blood in diabetes, it is stated to be unusual, as evidenced by a normal freezing-point; and the "not" seems to have been omitted. In the description of a case of diabetic coma on page 135, the figure for ammonia nitrogen evidently contains a misplaced decimal point. The reviewer would differ with the statement under Symptomatology that rapid wasting is readily explained by the loss of sugar through the urine, for he believes that loss of water is the chief cause of rapid loss of weight. He also believes that fever, rather than a subnormal temperature, is common in coma.

Foster's dicta as to prognosis are based on considerations which seem to the reviewer incontestable in the main, the age of the patient, rather than the severity of the disease existing at the moment, being given the greatest weight. He would, however, feel that the statement that all children succumb within a comparatively short period is too sweeping. Cases of protracted duration of a mild type of diabetes are known to exist in children, and the

reviewer has personal knowledge of them. Such cases were reported, for instance, by Landahl. The explanation of the disappearance of sugar from the urine in older patients with hypertension leaves one without a clear idea of the author's real views. Similarly, in discussing the total metabolism, while giving the views of Benedict, Joslin and others, he does not indicate his own belief. He does, however, quite plainly express his opinion that some of the severest diabetics show the dextrose ratio of 3.65 to 1, on which Lusk has always laid stress. In the treatment of coma by intravenous infusion of alkali, no allusion is made to the use of sodium bicarbonate, made by passing CO_2 through the solution. Magnus-Levy has shown clearly the value of this. The discussion of the treatment of the surgical complications of diabetes is excellent for so short a book, and the advice given seems very wise, and based on sound clinical observation. The final chapter on the identification of sugars is a useful accompaniment of the book, but it is not very intelligibly presented.

The volume may be recommended to the physician who is not a specialist in the chemical side of medicine as a sane statement of existing knowledge and a sound basis for practice.

A CORRECTION

Attention is called to an error on page 7 of THE ARCHIVES for January in the article by Dr. Frank A. Evans, "Observations on the Origin and Status of the So-Called 'Transitional' White Blood Cell." The last sentence of the legend of Figure 2 should read: "Solid line above, polymorphonuclears; line with crosses, transitionals; line with dots, large mononuclears." The error consisted in a transposition of the descriptions of the lines with crosses and dots, giving an erroneous idea of what the author intended.